

12/23/99

jc675 U.S. PTO



12-217-91

Box Sec 1A

Docket No. 59896/JPW/ADM

jc525 U.S. PTO
09/471572

12/23/99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

December 23, 1999

S I R:

Transmitted herewith for filing are the specification and claims of the patent application of:

Kenneth A. Jones, Mary W. Walker, Joseph Tamm, Theresa A. Branchek and _____ for
Inventor(s) Christophe P.G. Gerald

CHIMERIC G PROTEINS AND USES THEREOF
Title of Invention

Also enclosed are:

- ☒ 8 sheet(s) of _____ informal ☒ formal drawings.
- ☒ Oath or declaration of Applicant(s).
- ☒ A power of attorney
- ☒ An assignment of the invention to Synaptic Pharmaceutical Corporation
- ☒ A Preliminary Amendment
- ☒ A verified statement to establish small entity status under 37 C.F.R. §1.9 and §1.27.

The filing fee is calculated as follows:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE		FEE	
					SMALL ENTITY	OTHER ENTITY	SMALL ENTITY	OTHER ENTITY
TOTAL CLAIMS	24 -20	=	4	X	\$ 9	\$ 18	= \$ 36	\$
Independent Claims	3 -3	=	0	X	\$ 39	\$ 78	= \$ 0	\$
Multiple Dependent Claims Presented: _____ Yes <input checked="" type="checkbox"/> No					\$ 130	\$260	= \$ 0	\$
					BASIC FEE		\$ 380	\$
					TOTAL FEE		\$ 416	\$

* If the difference in Col. 1 is less than zero, enter "0" in Col. 2

Applicants: Kenneth A. Jones et al.
Serial No.: Not Yet Known
Filed: Herewith

Letter of Transmittal
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☒ A check in the amount of \$ 416.00 to cover the filing fee.

☐ Please charge Deposit Account No. _____ in the amount of \$ _____.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required in connection with the following or credit any over-payment to Account No. 03-3125;

☒ Filing fees under 37 C.F.R. §1.16.

☒ Patent application processing fees under 37 C.F.R. §1.17.

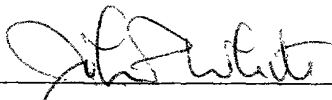
☐ The issue fee set in 37 C.F.R. §1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. §1.311(b).

☒ Three copies of this sheet are enclosed.

☐ A certified copy of previously filed foreign application No. _____ filed in _____ on _____ Applicant(s) hereby claim priority based upon this aforementioned foreign application under 35 U.S.C. §119.

☒ Other (identify) Express Mail Certificate of Mailing bearing the label No. EL066381980US. dated December 23, 1999 duplicate set of drawings. Sequence Listing. Statement in Accordance With 37 C. F. R. §1.821 (f). computer diskette containing Sequence Listing

Respectfully submitted,



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(c) An independent inventor as used in this chapter means any inventor who (1) has not assigned, granted, conveyed, or licensed, and (2) is under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who could not likewise be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern or a nonprofit organization under this section.

(d) A small business concern as used in this chapter means any business concern as defined by the Small Business Administration in 13 C.F.R. §121.3-18, published on September 30, 1982 at 47 FR 43273. For the convenience of the users of these regulations, that definition states:

§121.3-18 Definition of small business for paying reduced patent fees under Title 35, U.S. Code.

(a) Pursuant to Pub. L. 97-247, a small business concern for purposes of paying reduced fees under 35 U.S. Code 41(a) and (b) to the Patent and Trademark Office means any business concern (1) whose number of employees, including those of its affiliates, does not exceed 500 persons and (2) which has not assigned, granted, conveyed, or licensed, and is under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern or a nonprofit organization under this section. For the purpose of this section concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both. The number of employees of the business concern is the average over the fiscal year of the the persons employed during each of the pay periods of the fiscal year. Employees are those persons employed on a full-time, part-time or temporary basis during the previous fiscal year of the concern.

(b) If the Patent and Trademark Office determines that a concern is not eligible as a small business concern within this section, the concern shall have a right to appeal that determination to the Small Business Administration. The Patent and Trademark Office shall transmit its written decision and the pertinent size determination file to the SBA in the event of such adverse determination and size appeal. Such appeals by concerns should be submitted to the SBA at 1441 L Street, NW., Washington, D.C. 20416 (Attention: SBA Office of General Counsel). The appeal should state the basis upon which it is claimed that the Patent and Trademark Office initial size determination on the concern was in error; and the facts and arguments supporting the concern's claimed status as a small business concern under this section.

(e) A nonprofit organization as used in this chapter means (1) a university or other institution of higher education located in any country; (2) an organization of the type described in section 501(c)(3) of the Internal Revenue Code of 1954 (26 U.S.C. 501(c)(3)) and exempt from taxation under section 501(a) of the Internal Revenue Code (26 U.S.C. 501(a)); (3) any nonprofit scientific or educational organization qualified under a nonprofit organization statute of a state of this country (35 U.S.C. 201(i)); or (4) any nonprofit organization located in a foreign country which would qualify as a nonprofit organization under paragraphs (e)(2) or (3) of this section if it were located in this country.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. 37 C.F.R. §1.28(b)*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Kathleen P. Mullinix

Title In Organization: President and Chief Executive Officer

Address: 215 College Road
Paramus, New Jersey 07652

Signature: Kathleen P. Mullinix

Date Of Signature: December 20, 1999

*See Reverse

(b) Once status as a small entity has been established in an application or patent, fees as a small entity may thereafter be paid in that application or patent without regard to a change in status until the issue fee is due or any maintenance fee is due. Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application or patent prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate pursuant to §1.9 of this part. The notification of change in status may be signed by the applicant, any person authorized to sign on behalf of the assignee, or an attorney or agent of record or acting in a representative capacity pursuant to §1.34(a) of this part.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Kenneth A. Jones, Mary W. Walker,
Joseph Tamm, Theresa A. Brancheck, and
Christophe P.G. Gerald

U.S. Serial No. : Not Yet Known

Filed : Herewith

For : CHIMERIC G PROTEINS AND USES THEREOF

1185 Ave of the Americas
New York, New York 10036
December 23, 1999

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please amend the subject application as follows:

In the claims:

Please cancel claims 23-76, 78-140, and 142-155 without prejudice to applicants' right to pursue the subject matter of these claims in a future continuation or divisional application.

REMARKS

Claims 1-155 were pending in the subject application. By this Amendment applicants have canceled claims 23-76, 78-140, and 142-155 without prejudice or disclaimer. Accordingly, claims 1-22, 77, and 141 are currently pending.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

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No fee, other than the enclosed fee of \$416.00 for filing this application, is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully Submitted,

A handwritten signature in dark ink, appearing to read "John P. White", is written over a horizontal line.

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*Application
for
United States Letters Patent*

To all whom it may concern:

Be it known that **Kenneth A. Jones, Mary W. Walker, Joseph Tamm, Theresa A. Branchek,
and Christophe P.G. Gerald**

have invented certain new and useful improvements in

Chimeric G Proteins And Uses Thereof

of which the following is a full, clear and exact description.

CHIMERIC G PROTEINS AND USES THEREOF

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BACKGROUND OF THE INVENTION

10 Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by
15 reference into this application in order to more fully describe the state of the art to which the invention pertains.

Therapeutic importance of G protein-coupled receptors

20 Intercellular communication in multicellular organisms relies on numerous signal transduction pathways that allow chemical messages to be sensed extracellularly and converted into intracellular
25 responses. One of the most ancient and well-diversified pathways uses G protein-coupled receptors (GPCRs) as the chemical sensor. GPCRs comprise a large family of transmembrane signaling proteins that are key to a variety of cellular activities including
30 phototransduction, olfaction, neurotransmission, and endocrine function.

There are currently about 300 molecularly identified GPCRs and this number is rapidly growing. Estimates
35 based on genomes that have been entirely sequenced suggest that there may be more than 1000 GPCRs in humans. The fact that a large proportion of prescribed drugs act on GPCRs coupled with the evidence of a large reserve of undiscovered genes

suggests that these proteins will continue to be major targets for drug discovery for the foreseeable future.

5 Signaling pathways used by GPCRs

GPCRs mediate diverse cellular responses to external stimuli through their interaction with a single class of proteins known as heterotrimeric G proteins (G proteins). These proteins are composed functionally of two subunits, an α subunit that possesses GPCR-recognition and GTP-binding domains, and a dimer formed by β and γ subunits (Bourne, 1997; Lambright et al., 1996). Stimulated by agonist binding, GPCRs induce a conformational change in the G protein that facilitates the exchange of GDP for GTP bound to the α subunit. In the GTP-bound state, the α subunit is free to dissociate from the $\beta\gamma$ dimer, permitting the two subunits to independently interact with a number of membrane-bound effector proteins including enzymes and ion channels.

To date, there are 17 $G\alpha$ subunits that have been cloned (Simon et al., 1991). These fall broadly into four classes: those that activate phospholipase C ($G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$), those that stimulate adenylate cyclase ($G\alpha_s$ and isoforms), those that mediate inhibition of adenylate cyclase and also permit interaction with a variety of other effectors through release of $\beta\gamma$ subunits ($G\alpha_i$ and $G\alpha_o$ isoforms), and finally $G\alpha_{12}$ and $G\alpha_{13}$ whose regulatory functions are less well understood. By detecting and discriminating among structural features of both $\beta\gamma$ and $G\alpha$, the individual GPCR activates only a subset of available G proteins (Bourne, 1997).

The "funneling" of signaling events through specific classes of G proteins has had important consequences for the design of assays to test the functional status of a given receptor. For example, receptors that couple strongly to $G\alpha_q$, such as α_{1A} -adrenoceptors, 5-HT_{2C} receptors, or H1 histamine receptors, activate phospholipase C isoforms, initiating a rise in inositol phosphates (IP3) and a release of calcium from intracellular stores. Specific assays have been developed to measure the release of these signaling molecules. Likewise, other assays have been developed for measuring accumulation or depletion of cAMP (from stimulation or inhibition of adenylate cyclase) due to stimulation of receptors coupling either to $G\alpha_s$ or $G\alpha_i$, respectively. A myriad of other assays have been elaborated that measure ion channel, GTPyS binding, MAP kinase, or transcriptional activities. In further elaborations of these methods, artificial "reporter genes" are used to provide a simplified endpoint initiated by some of the above cellular responses.

Ligand identification for GPCR "orphan" receptors

The discovery of new GPCRs has outpaced the identification of new natural ligands, leading to a growing list of "orphan" G protein-coupled receptors whose ligand is unknown. Identifying the ligands for these orphan receptors is critical for determining their biological importance and will permit investigations into receptor pharmacology and drug design. While it is possible to identify ligands by binding, such assays depend upon the availability of high affinity radiolabeled ligands, and often on high levels of expression of the cloned receptor. On the

other hand, functional activity can be elicited using unmodified, naturally occurring ligands applied to cells expressing moderate densities of receptor. The primary disadvantage of the functional approach is not knowing which class of G protein will couple efficiently to the orphan receptor. Although much progress has been made toward identifying motifs within the intracellular portions of GPCRs that bind G proteins, currently it is not possible to predict which class of G protein will couple to a given receptor. This uncertainty requires the employment of multiple functional assays for each orphan receptor in order to cover all possible signal transduction pathways. The availability of a single, genetically modified G protein that could couple universally to the vast majority of GPCRs would be an extremely useful tool for the study of orphan receptors and for the development of new therapeutic agents targeting GPCRs.

"Promiscuous" G proteins and modified G proteins

The design of a universal functional assay for all GPCRs is a highly sought after goal for the pharmaceutical industry. Such an assay would eliminate the need to run multiple parallel assays for each receptor. Work on the $G\alpha_{16}$ subunit (Offermans and Simon, 1995) showed that a single G protein can "route" receptors that normally couple to inhibition of adenylate cyclase to stimulation of inositol phosphate production (Offermanns and Simon, 1995). Such a system can take advantage of instrumentation that detects Ca^{++} mobilization via fluorescent dyes in a multiwell plate format suitable for mass screening of compound libraries. Unfortunately, while heterologous expression systems

incorporating $G\alpha_{16}$ are amenable to mass screening, there are a significant number of GPCRs that do not couple well to this G protein, reducing its general utility for screening orphan receptors.

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Studies of the three dimensional structure of native G proteins (Lambright et al., 1996) and the functional activities of chimeric G proteins (see for review, Milligan and Rees, 1999) point to two regions of the $G\alpha$ subunit that are involved in receptor recognition. Conklin and co-workers (Conklin et al., 1993) provided experimental evidence that the extreme C-terminal regions of $G\alpha_q$, $G\alpha_s$, and $G\alpha_{12}$ are important for directing targeting to the receptor. For example, replacing the last five amino acids of $G\alpha_q$ with the corresponding amino acids from $G\alpha_{12}$, permitted three receptors, which normally couple to $G\alpha_{1/o}$, to stimulate phospholipase C (PLC). Similarly, replacing with the terminal five amino acids of $G\alpha_s$, permitted stimulation of PLC by the vasopressin V2 receptor, which normally activates adenylate cyclase (Conklin et al., 1996). Other experiments, in which $G\alpha_s$ was altered by the C-terminal amino acids of $G\alpha_q$, demonstrated the generality of the finding that a given G protein can be re-directed by replacing the C-terminus of a given $G\alpha$ "backbone" with the appropriate C-terminus of another $G\alpha$ subunit (see for review, Milligan and Rees, 1999). Thus, the C-terminus of $G\alpha$ is one important determinant for GPCR recognition and may be modified to channel responses from the preferred signaling pathway to another one that would be amenable to automation.

The N-terminus of $G\alpha$ is also involved in directing G protein to a target receptor, but the specificity for

35

this is much less well understood. Kostenis and co-workers (Kostenis et al., 1997; Kostenis et al., 1998) noted that the N-termini of $G\alpha_q$ and $G\alpha_{11}$ are unique in that they contain a six amino acid extension not found in other $G\alpha$ subunits. Deletion of this extension permitted GPCRs that do not normally couple to wild-type $G\alpha_q$, to productively couple to the mutant and activate PLC. Although N-terminal deletion mutants of $G\alpha_q$ improve coupling to $G\alpha_{1/o}$ -coupled receptors, the amplitude of second messenger response in many instances is low and not sufficient for mass screening applications.

Use of ancestral G proteins

Sequence analysis of $G\alpha$ genes from organisms spanning multiple phyla suggests the existence of a primordial $G\alpha$ ancestor (Wilkie and Yokoyama, 1994; Seack et al., 1998; Suga et al., 1999; Figure 1). Lower organisms having less elaborate second messenger pathways and effector protein targets might harbor $G\alpha$ homologues that are closer in structure to the ancestral protein. Further, these proteins may have the capacity to interact promiscuously with a wide variety of GPCRs because they lack structural motifs that subsequently evolved for the recognition of specific receptor subtypes. For example, in the search for primitive G proteins we noted that all invertebrate species, including *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster* (*D. melanogaster*), lack the first six amino acids corresponding to the N-terminus of mammalian $G\alpha_q$ subunits. The use of $G\alpha$ subunits from species that appear evolutionarily early on the phylogenetic tree offers an approach to universal coupling that has not been previously described.

C. elegans is an attractive organism because its genome has been completely sequenced (The *C. elegans* Sequencing Consortium, 1998) and because, as a pseudocoelomate, it branches early in the phylogenetic tree (Keeton, 1980). *C. elegans* contains only a single homologue from each of the four major $G\alpha$ families: $G\alpha_q$, $G\alpha_i$, $G\alpha_s$, and $G\alpha_{12}$ (Jansen et al., 1999). This contrasts with mammals which have multiple isoforms within each of these families and, at the other phylogenetic extreme, yeast which has only two $G\alpha$ subunits (Simon et al., 1991). The single $G\alpha_q$ subunit of *C. elegans* may, therefore, couple to a wider range of GPCRs than any of its mammalian homologues. When combined with specific C-terminal tails derived from mammalian non- $G\alpha_q$ subunits, the resulting chimeric G proteins may be further enhanced in their ability to efficiently couple to mammalian GPCRs.

This application describes the use of $G\alpha_q$ subunits obtained from invertebrate organisms, using *C. elegans* and *D. melanogaster* as examples, as "backbones" for the construction of chimeras. One chimera in particular, composed of *C. elegans* $G\alpha_q$ ($cG\alpha_q$) and modified to contain on its C-terminus the five amino acids of human $G\alpha_z$ ($hG\alpha_z$), exhibits surprisingly robust coupling to 78% of a large sample of cloned GPCRs. Further described are uses for this $G\alpha$ chimera, and others, related to the identification of ligands for orphan GPCRs and for high-throughput screening of chemical compounds in functional assays.

SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid encoding a chimeric G protein, wherein the chimeric G protein comprises an invertebrate G α q G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than five amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a

mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

In addition, the invention provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as

to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

5 The invention also provides a process for determining whether a chemical compound specifically binds to and activates a mammalian G protein-coupled receptor, which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing
10 the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence
15 of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor.

20 In addition, the invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian G protein-coupled receptor, which comprises separately
25 contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G
30 protein, with both the chemical compound and a second chemical compound known to activate the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian G protein-coupled
35 receptor, and measuring the second messenger response in the presence of only the second chemical compound

and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a compound which activates the mammalian G protein-coupled receptor which comprises:

- (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;
- (b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so
- (c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor.

The invention still further provides a process of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian G protein-coupled receptor to identify a compound which inhibits the activation of the mammalian G protein-coupled receptor, which comprises:

- (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting activation of the mammalian G protein-coupled receptor;
- (b) determining whether the extent or amount of activation of the mammalian G protein-coupled receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian G protein-coupled receptor in the absence of such one or more compounds; and if so
- (c) separately determining whether each such compound inhibits activation of the mammalian G protein-coupled receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises separately

contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [³⁵S]GTPγS, and with only [³⁵S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [³⁵S]GTPγS binding to the membrane preparation and an increase in [³⁵S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

In addition, the invention provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound, [³⁵S]GTPγS, and a second chemical compound known to activate the mammalian G protein-coupled receptor, with [³⁵S]GTPγS and only the second compound, and with [³⁵S]GTPγS alone, under conditions permitting the activation of the mammalian G protein-coupled receptor, detecting [³⁵S]GTPγS binding to each membrane preparation, comparing the increase in [³⁵S]GTPγS binding in the presence of the compound and the second compound relative to the binding of [³⁵S]GTPγS alone to the increase in [³⁵S]GTPγS binding in the presence of the second chemical compound relative to the binding of [³⁵S]GTPγS alone, and detecting a smaller increase in [³⁵S]GTPγS binding in the presence of the compound and the second compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

The inventions still further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

The invention also provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises

contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

10 The invention further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a
15 chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting
20 specific binding of the chemical compound to the mammalian G protein-coupled receptor.

In addition, the invention provides a process involving competitive binding for identifying a
25 chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled
30 receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the mammalian G protein-coupled receptor, and with only the second chemical compound,
35 under conditions suitable for binding of both compounds, and detecting specific binding of the

chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention also provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

- (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing

DNA encoding a mammalian G protein-coupled receptor with a compound known to bind specifically to the mammalian G protein-coupled receptor;

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(b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor, under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

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(c) determining whether the binding of the compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

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(d) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

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The invention further provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

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(a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the

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plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

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(b) determining whether the binding of a compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

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(c) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

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The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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The invention further provides a process for determining whether a chemical compound is a ligand

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for a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention still further provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

In addition, the invention provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a ligand for the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

10

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

15

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [³⁵S]GTPγS, and with only [³⁵S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [³⁵S]GTPγS binding to the membrane preparation and an increase in [³⁵S]GTPγS binding in the presence of the

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compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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In addition, the invention provides a process for determining whether a chemical compound is a ligand for the mammalian G protein-coupled receptor, which comprises contacting cells transfected with and
10 expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in
15 receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled
20 receptor.

The invention further provides a process for identifying a ligand for a mammalian G protein-coupled receptor which comprises contacting cells
25 transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under
30 conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled
35 receptor.

The invention still further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from
5 cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under
10 conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

15 The invention also provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a
20 mammalian G protein-coupled receptor, which comprises:

- 25 (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting activation of a mammalian G protein-coupled receptor;
- 30 (b) determining whether the ligand activates the cells expressing the plurality of independent clones and the chimeric G protein; and if so
- 35 (c) isolating the single clone which expresses the mammalian G protein-coupled receptor activated by the ligand, so as to thereby identify any

clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

5 The invention further provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor,
10 which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones
15 with a ligand, under conditions permitting specific binding to a mammalian G protein-coupled receptor;

(b) determining whether the ligand specifically
20 binds to the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the
25 mammalian G protein-coupled receptor which specifically binds to the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

BRIEF DESCRIPTION OF THE FIGURES**Figure 1**

Phylogenetic tree of the $G\alpha_q$ family of G proteins.
 5 The tree was created using the "Growtree" algorithm
 and specifying the "Cladogram" output (SeqLab version
 10, Genetics Computer Group). Initially, a cladogram
 was created from a multiple sequence alignment
 ("Pileup" utility in SeqLab version 10, Genetics
 10 Computer Group) of all publicly available G protein
 sequences. The tree was then edited for clarity by
 removing non- $G\alpha_q$ sequences. Double tilde indicates a
 break in the branch to *Dictyostelium discoideum*
 sequences imposed to permit page formatting. Branch
 15 lengths are proportional to the number of accumulated
 amino acid substitutions.

GBA2_DICDI is *Dictyostelium discoideum* $G\alpha_2$ (Genbank
 Accession number P16051); GBA4_DICDI is *Dictyostelium*
 20 *discoideum* $G\alpha_4$ (Genbank Accession number P34042);
 GB16_MOUSE is *Mus musculus* (mouse) $G\alpha_{16}$ (Genbank
 Accession number G193571); GB16_HUMAN is *Homo sapiens*
 (human) $G\alpha_{16}$ (Genbank Accession number G182892);
 GBQ_PATYE is *Patinopecten yessoensis* $G\alpha_q$ (GenBank
 25 Accession number O15975); GBQ_LYMST is *Lymnaea*
stagnalis $G\alpha_q$ (GenBank Accession number P38411);
 GBQ_HUMAN is *Homo sapiens* (human) $G\alpha_q$ (Genbank
 Accession number L76256); GBQ_CANFA is *Canis*
familiarus $G\alpha_q$ (Genbank Accession number Q28294);
 30 GBQ_MOUSE is *Mus musculus* (mouse) $G\alpha_q$ (Genbank
 Accession number P21279); GBQ_XENLA is *Xenopus laevis*
 $G\alpha_q$ (Genbank Accession number P38410); GB11_HUMAN is
Homo sapiens (human) $G\alpha_{11}$ (Genbank Accession number
 29992); GB11_BOVIN is *Bos taurus* (bovine) $G\alpha_{11}$

(Genbank Accession number P38409); GB11_MOUSE is *Mus musculus* (mouse) $G\alpha_{11}$ (Genbank Accession number P21278); GB11_MELGA is *Meleagris gallopavo* $G\alpha_{11}$ (Genbank Accession number P45645); GB11_XENLA is *Xenopus laevis* $G\alpha_{11}$ (Genbank Accession number P43444); GBQ3_DROME is *Drosophila melanogaster* $G\alpha_{q3}$ (GenBank Accession number P54400); GBQ1_DROME is *Drosophila melanogaster* $G\alpha_{q1}$ (GenBank Accession number P23625); GBQ_HOMAM is *Homarus americanus* $G\alpha_q$ (GenBank Accession number P91950); GBQ_CAEEL is *Caenorhabditis elegans* $G\alpha_q$ (GenBank Accession number AF003739); GBQ_LOLFO is *Loligo forbesi* $G\alpha_q$ (GenBank Accession number P38412); GB14_MOUSE is *Mus musculus* (mouse) $G\alpha_{14}$ (Genbank Accession number P30677); GB14_BOVIN is *Bos taurus* (bovine) $G\alpha_{14}$ (Genbank Accession number P38408); and GBQ_GEOCY is *Geodia cydonium* $G\alpha_q$ (GenBank Accession number γ 14248).

Figure 2A-2B

Amino acid sequences of $G\alpha_{q/x}$ chimeras. (*C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); and *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41)). Bold regions at the C-terminus denote where amino acid substitutions are made between *C. elegans* $G\alpha_q$ and mammalian $G\alpha_z$, $G\alpha_s$, or $G\alpha_{13}$. The remainder of the protein (non-bold amino acids) in each case is *C. elegans* or *D. melanogaster* $G\alpha_q$.

Figure 3

Examples of receptor-evoked responses in oocytes expressing c $G\alpha_{q/z5}$ or h $G\alpha_{q/z5}$ chimeric G proteins.

Figure 4

Examples of receptor-evoked responses in mammalian cells expressing cG $\alpha_{q/z5}$ or hG $\alpha_{q/z5}$ chimeric G proteins plus the human D1 receptor. Transiently transfected COS-7 cells were seeded into a 96-well microtiter plate and monitored for calcium mobilization in the FLIPR™ using the calcium-sensitive dye Fluo-3. A) Representative time course of fluorescence in cells stimulated at time = 10 seconds with 100 μ M dopamine. Each curve is derived from a representative well. B) Maximal change in relative fluorescent units was calculated for dopamine concentrations ranging from 0.3 nM to 100 μ M. Triplicate determinations, plotted as mean \pm standard error of the mean, were used to construct concentration-response curves. In the example shown here, a measurable response to dopamine was obtained only in the presence of cG $\alpha_{q/z5}$, with a maximal signal of 14,723 fluorescence units and pEC₅₀ of 6.32. Average maximal responses from multiple experiments (n \geq 2) are listed in Table 5.

Figures 5A-5C

Multiple sequence alignment of G α_q proteins from invertebrate and vertebrate organisms. Sequences were aligned using "Pileup" (SeqLab version 10, Genetics Computer Group). The degree of amino acid identity is indicated by the level of shading (black, 100% identity, white < 60%).

GBQ_HUMAN is *Homo sapiens* (human) G α_q (Genbank Accession number L76256; SEQ ID NO: 6); GBQ_CANFA is *Canis familiaris* G α_q (Genbank Accession number Q28294; SEQ ID NO: 7); GBQ_MOUSE is *Mus musculus* (mouse) G α_q (Genbank Accession number P21279; SEQ ID NO: 8);

GBQ_XENLA is *Xenopus laevis* $G\alpha_q$ (Genbank Accession
 number P38410; SEQ ID NO: 9); GBQ_PATYE is
Patinopecten yessoensis $G\alpha_q$ (GenBank Accession number
 O15975; SEQ ID NO: 10); GBQ_LYMST is *Lymnaea*
 5 *stagnalis* $G\alpha_q$ (GenBank Accession number P38411; SEQ ID
 NO: 11); GBQ1_DROME is *Drosophila melanogaster* $G\alpha_{q1}$
 (GenBank Accession number P23625; SEQ ID NO: 12);
 GBQ3_DROME is *Drosophila melanogaster* $G\alpha_{q3}$ (GenBank
 Accession number P54400; SEQ ID NO: 13); GBQ_HOMAM is
 10 *Homarus americanus* $G\alpha_q$ (GenBank Accession number
 P91950; SEQ ID NO: 14); GBQ_LIMPO is *Limulus*
polyphemus $G\alpha_q$ (Genbank Accession number g1857923; SEQ
 ID NO: 15); GBQ_LOLFO is *Loligo forbesi* $G\alpha_q$ (GenBank
 Accession number P38412; SEQ ID NO: 16); GBQ_CAEEL is
 15 *Caenorhabditis elegans* $G\alpha_q$ (GenBank Accession number
 AF003739; SEQ ID NO: 17); GBQ_GEOCY is *Geodia*
cydonium $G\alpha_q$ (GenBank Accession number 14248; SEQ ID
 NO: 18).

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are presented as an aid in understanding this invention.

5

For the purposes of this invention, "ligand" is a molecule capable of binding to and modulating a receptor. The ligand may be chemically synthesized or may occur in nature.

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For the purposes of this invention, "agonist" is a ligand capable of stimulating the biological activity of a receptor.

15

For the purposes of this invention, "antagonist" is a ligand capable of inhibiting the biological activity of a receptor.

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For the purposes of this invention, "invertebrate" species are defined as those members of the Animal Kingdom that do not possess a vertebral column or backbone (Barnes, 1974).

25

For the purposes of this invention, in one embodiment, an invertebrate Gαq G protein has amino acids QK at positions 12 and 13 from the N-terminus and does not contain the sequence MTLESI (SEQ ID NO: 36) at the N-terminus.

30

For the purposes of this invention, "vertebrate" species are those members of the Animal Kingdom that do possess a vertebral column or backbone (Barnes, 1974). A common characteristic of vertebrate Gαq G proteins is an N-terminal extension composed of the amino acids MTLESI (SEQ ID NO: 36).

35

For the purposes of this invention, "G α q second messenger response" is one of a number of responses which are typically produced by activation of G protein heterotrimers containing G α q.

For the purposes of this invention, "G α s second messenger response" is one of a number of responses which are typically produced by activation of G protein heterotrimers containing G α s.

For the purposes of this invention, "receptor/G protein heterotrimer association/ dissociation" means a change in the intermolecular relationship between either α - β - γ subunits themselves or one or more of these subunits with the receptor.

Having due regard to the preceding definitions, the present invention provides an isolated nucleic acid encoding a chimeric G protein, wherein the chimeric G protein comprises an invertebrate G α q G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than five amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

In one embodiment, the nucleic acid encodes a chimeric G protein, wherein the chimeric G protein

comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than two amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

In another embodiment, the nucleic acid encodes a chimeric G protein, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

In one embodiment, the nucleic acid is DNA. In one embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another embodiment, the nucleic acid is RNA.

In one embodiment, the aforementioned vertebrate G protein is a mammalian G protein. In another embodiment, the aforementioned contiguous amino acids which have been deleted are contained in FVFAAVKDTILQHNLKEYNLV* (SEQ ID NO: 37), wherein V* is the C-terminal amino acid.

In another embodiment, the vertebrate G protein is a vertebrate G α z G protein. In another embodiment, the number of contiguous amino acids which have replaced the deleted amino acids are contained in
 5 FVFDAVTDVIIQNNLKYIGLC* (SEQ ID NO: 38), wherein C* is the C-terminal amino acid. In another embodiment, the aforementioned invertebrate G α q G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and
 10 replaced by five contiguous amino acids beginning with the C-terminal amino acid of the vertebrate G α z protein.

In another embodiment, the vertebrate G protein is a vertebrate G α s G protein. In another embodiment, the number of contiguous amino acids which have replaced the deleted amino acids are contained in
 15 RVFNDCRDIIQRMHLRQYELL* (SEQ ID NO: 39), wherein L* is the C-terminal amino acid. In another embodiment, the invertebrate G α q G protein has nine contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by nine
 20 contiguous amino acids beginning with the C-terminal amino acid of the vertebrate G α s protein.

25 In another embodiment, the vertebrate G protein is a vertebrate G α i3 G protein. In another embodiment, the number of contiguous amino acids which have replaced the deleted amino acids are contained in
 30 FVFDAVTDVIIKNNLKECGLY* (SEQ ID NO: 40), wherein Y* is the C-terminal amino acid. In another embodiment, the invertebrate G α q G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five

contiguous amino acids beginning with the C-terminal amino acid of the vertebrate $G\alpha i3$ protein.

5 In other embodiments, the vertebrate G protein is a vertebrate $G\alpha i1$ G protein, a vertebrate $G\alpha i2$ G protein, a vertebrate $G\alpha oA$ G protein, or a vertebrate $G\alpha oB$ G protein.

10 In another embodiment, the invertebrate $G\alpha q$ G protein is a *Caenorhabditis elegans* $G\alpha q$ G protein. In still other embodiments, the invertebrate $G\alpha q$ G protein is a *Drosophila melanogaster* $G\alpha q$ G protein, a *Limulus polyphemus* $G\alpha q$ G protein, a *Patinopecten yessoensis* $G\alpha q$ G protein, a *Loligo forbesi* $G\alpha q$ G protein, a
15 *Homarus americanus* $G\alpha q$ G protein, a *Lymnaea stagnalis* $G\alpha q$ G protein, a *Geodia cydonium* $G\alpha q$ G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein.

20 In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO:
25 4); (e) Figure 2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

30 The invention provides a vector comprising any of the aforementioned nucleic acids. In different embodiments, the vector is adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding

the chimeric G protein so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect, or mammalian cell. In different embodiments, the vector is a plasmid, a baculovirus, or a retrovirus.

The invention provides a cell comprising any of the aforementioned vectors, wherein the cell comprises DNA encoding a mammalian G protein-coupled receptor. In one embodiment of the cell, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell. In one embodiment, the cell is a non-mammalian cell. In different embodiments, the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell. In another embodiment, the cell is a mammalian cell. In different embodiments, the mammalian cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell. In one embodiment, the cell is an insect cell. In different embodiments, the insect cell is an Sf9 cell, an Sf21 cell or a *Trichoplusia ni* 5B-4 cell. The invention provides a membrane preparation isolated from any of the aforementioned cells.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled

receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

5 The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a
10 chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled
15 receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

The invention also provides a process for determining
20 whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with
25 the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as
30 to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

The invention further provides a process for
35 determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting a membrane preparation from

cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

In one embodiment of any of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell. In different embodiments, the mammalian G protein-coupled receptor is a human Y5 receptor, a human GALR2 receptor, a human kappa opioid receptor, a human NPFF1 receptor, a human NPFF2 receptor, a human α 2A adrenergic receptor, a human dopamine D2 receptor, a human GALR1 receptor, a human Y2 receptor, a human Y1 receptor, a human Y4 receptor, a human α 1A adrenergic receptor, a human dopamine D1 receptor, or a rat NTR1 receptor.

The invention also provides a process for determining whether a chemical compound specifically binds to and activates a mammalian G protein-coupled receptor, which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G

protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor. In one embodiment of the process, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of the aforementioned process, the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element. In another embodiment, the second messenger response is measured by a change in cell proliferation. In another embodiment, the second messenger response is a $G\alpha_q$ second messenger response. In one embodiment, the $G\alpha_q$ second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate. In another embodiment, the $G\alpha_q$ second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid. In another embodiment, the $G\alpha_q$ second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation. In another embodiment, the $G\alpha_q$ second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In one embodiment, the measure of intracellular calcium levels is made by

chloride current readings. In other embodiments, the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

In addition, the invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian G protein-coupled receptor, which comprises separately contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to activate the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian G protein-coupled receptor. In one embodiment of the process, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, DNA encoding the mammalian G

protein-coupled receptor is transfected into the cell.

5 In one embodiment of the aforementioned process, the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element. In another embodiment, the second messenger response is measured by a change in cell proliferation. In another embodiment, the
10 second messenger response is a $G\alpha_q$ second messenger response. In one embodiment, the $G\alpha_q$ second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol
15 phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the $G\alpha_q$ second messenger response comprises activation of MAP kinase and the change in
20 second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the $G\alpha_q$
25 second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the
30 presence of only the second chemical compound. In another embodiment, the $G\alpha_q$ second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in
35 the presence of both the chemical compound and the

second chemical compound than in the presence of only the second chemical compound. In one embodiment, the measure of intracellular calcium levels is made by chloride current readings. In other embodiments, the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

The invention also provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a compound which activates the mammalian G protein-coupled receptor which comprises:

- (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;
- (b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so
- (c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby

identify each compound which activates the mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian G protein-coupled receptor to identify a compound which inhibits the activation of the mammalian G protein-coupled receptor, which comprises:

10

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting activation of the mammalian G protein-coupled receptor;

15

(b) determining whether the extent or amount of activation of the mammalian G protein-coupled receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian G protein-coupled receptor in the absence of such one or more compounds; and if so

25

(c) separately determining whether each such compound inhibits activation of the mammalian G protein-coupled receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian G protein-coupled receptor.

35

In one embodiment of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [³⁵S]GTPγS, and with only [³⁵S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [³⁵S]GTPγS binding to the membrane preparation and an increase in [³⁵S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound, [³⁵S]GTPγS, and a second chemical compound known to activate the mammalian G protein-coupled receptor, with [³⁵S]GTPγS and only the second compound, and with [³⁵S]GTPγS alone, under conditions permitting the activation of the mammalian G protein-coupled receptor, detecting [³⁵S]GTPγS binding to each membrane preparation, comparing the

increase in [³⁵S]GTPγS binding in the presence of the compound and the second compound relative to the binding of [³⁵S]GTPγS alone to the increase in [³⁵S]GTPγS binding in the presence of the second chemical compound relative to the binding of [³⁵S]GTPγS alone, and detecting a smaller increase in [³⁵S]GTPγS binding in the presence of the compound and the second compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

In one embodiment of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell. In one embodiment, the mammalian G protein-coupled receptor produces a Gαs second messenger response in the absence of the chimeric G protein.

This invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

This invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

In one embodiment of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of any of the aforementioned processes, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the

C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_z$ protein, wherein

5 such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid

10 have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_s$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_s$ protein, wherein such number equals the number of amino acids deleted. In another

15 embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino

20 acids present in a vertebrate $G\alpha_{i3}$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_{i3}$ protein, wherein such number equals the number of amino acids deleted.

25 In another embodiment, the chimeric G protein comprises a *Caenorhabditis elegans* $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number

30 of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a

35 *Drosophila melanogaster* $G\alpha_q$ G protein, a *Limulus*

polyphemus $G\alpha_q$ G protein, a *Patinopecten yessoensis*
G\alpha_q G protein, a *Loligo forbesi* $G\alpha_q$ G protein, a
Homarus americanus $G\alpha_q$ G protein, a *Lymnaea stagnalis*
G\alpha_q G protein, a *Geodia cydonium* $G\alpha_q$ G protein, or a
5 *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at
least five, but not more than twenty-one, contiguous
amino acids beginning with the C-terminal amino acid
have been deleted and replaced by a number of
contiguous amino acids present in a vertebrate G
10 protein beginning with the C-terminal amino acid of a
vertebrate G protein, wherein such number equals the
number of amino acids deleted. In other embodiments,
the chimeric G protein has an amino acid sequence
substantially the same as the amino acid sequence
15 shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO:
1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2);
(c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d)
Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure
2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure
20 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

In one embodiment of any of the aforementioned
processes, the cell is an insect cell. In another
25 embodiment, the cell is a mammalian cell. In one
embodiment, the mammalian cell is nonneuronal in
origin. In further embodiments, the nonneuronal cell
is a COS-7 cell, 293 human embryonic kidney cell, a
CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a
30 LM(tk-) cell.

The invention also provides a process for identifying
a chemical compound which specifically binds to a
mammalian G protein-coupled receptor which comprises
35 contacting cells transfected with and expressing DNA

encoding a chimeric G protein and expressing DNA
encoding a mammalian G protein-coupled receptor,
wherein such cells do not normally express the DNA
encoding the chimeric G protein, with the compound
5 under conditions suitable for binding, and detecting
specific binding of the chemical compound to the
mammalian G protein-coupled receptor.

The invention further provides a process for
10 identifying a chemical compound which specifically
binds to a mammalian G protein-coupled receptor which
comprises contacting a membrane preparation from
cells transfected with and expressing DNA encoding a
chimeric G protein and expressing DNA encoding a
15 mammalian G protein-coupled receptor, wherein such
cells do not normally express the DNA encoding the
chimeric G protein, with the compound under
conditions suitable for binding, and detecting
specific binding of the chemical compound to the
20 mammalian G protein-coupled receptor.

In addition, the invention provides a process
involving competitive binding for identifying a
chemical compound which specifically binds to a
25 mammalian G protein-coupled receptor which comprises
separately contacting cells transfected with and
expressing DNA encoding a chimeric G protein and
expressing DNA encoding a mammalian G protein-coupled
receptor, wherein such cells do not normally express
30 the DNA encoding the chimeric G protein, with both
the chemical compound and a second chemical compound
known to bind to the mammalian G protein-coupled
receptor, and with only the second chemical compound,
under conditions suitable for binding of both
35 compounds, and detecting specific binding of the
chemical compound to the mammalian G protein-coupled

receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention also provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

- (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled

receptor with a compound known to bind specifically to the mammalian G protein-coupled receptor;

5 (b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor, under conditions permitting binding of compounds known to bind to the mammalian G
10 protein-coupled receptor;

(c) determining whether the binding of the compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the
15 plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian G protein-coupled receptor of each
20 compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

25

The invention further provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian
30 G protein-coupled receptor, which comprises

(a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a
35 mammalian G protein-coupled receptor with the plurality of compounds not known to bind

specifically to the mammalian G protein-coupled receptor under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

5

(b) determining whether the binding of a compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

10

(c) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

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In one embodiment of any of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

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In one embodiment of any of the aforementioned processes, the chimeric G protein comprises an invertebrate G α q G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the

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chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_z$ protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_s$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_s$ protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_{i3}$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_{i3}$ protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an *Caenorhabditis elegans* $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such

number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* $G\alpha_q$ G protein, a *Limulus polyphemus* $G\alpha_q$ G protein, a *Patinopecten yessoensis* $G\alpha_q$ G protein, a *Loligo forbesi* $G\alpha_q$ G protein, a *Homarus americanus* $G\alpha_q$ G protein, a *Lymnaea stagnalis* $G\alpha_q$ G protein, a *Geodia cydonium* $G\alpha_q$ G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

In one embodiment of any of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention further provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do

not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

In one embodiment of the aforementioned process, the second messenger response is a $G\alpha_q$ second messenger response. In one embodiment, the $G\alpha_q$ second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In one embodiment, the measure of intracellular calcium levels is made by chloride current readings. In other embodiments, the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

In addition, the invention provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a ligand for the mammalian G protein-coupled receptor which comprises:

- (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not

known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

5

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

10

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [³⁵S]GTPγS, and with only [³⁵S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [³⁵S]GTPγS binding to the membrane preparation and an increase in [³⁵S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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In addition, the invention provides a process for determining whether a chemical compound is a ligand for the mammalian G protein-coupled receptor, which comprises contacting cells transfected with and
 5 expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active
 10 state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor
 15 and is a ligand for the mammalian G protein-coupled receptor.

The invention further provides a process for identifying a ligand for a mammalian G protein-coupled receptor which comprises contacting cells
 20 transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the
 25 chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled
 30 receptor.

The inventions still further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which
 35 comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a

chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

In one embodiment of any of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of any of the aforementioned processes, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_z$ protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_s$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_s$ protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_{i3}$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_{i3}$ protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an *Caenorhabditis elegans* $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* $G\alpha_q$ G protein, a *Limulus polyphemus* $G\alpha_q$ G protein, a *Patinopecten yessoensis* $G\alpha_q$ G protein, a *Loligo forbesi* $G\alpha_q$ G protein, a *Homarus americanus* $G\alpha_q$ G protein, a *Lymnaea stagnalis* $G\alpha_q$ G protein, a *Geodia cydonium* $G\alpha_q$ G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at

least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

In one embodiment of any of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention also provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing

DNA encoding a plurality of independent clones with a ligand, under conditions permitting activation of a mammalian G protein-coupled receptor;

5

(b) determining whether the ligand activates the cells expressing the plurality of independent clones and the chimeric G protein; and if so

10

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor activated by the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

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The invention further provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

20

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting specific binding to a mammalian G protein-coupled receptor;

30

(b) determining whether the ligand specifically binds to the cells expressing the plurality of independent clones and the chimeric G protein; and if so

35

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor which specifically binds to the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

In one embodiment of the aforementioned processes, the DNA encoding the plurality of independent clones is endogenous to the cell. In another embodiment, the DNA encoding the plurality of independent clones is transfected into the cell.

In one embodiment of the aforementioned processes, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_z$ protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been

deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_s$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_s$ protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_{i3}$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_{i3}$ protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an *Caenorhabditis elegans* $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* $G\alpha_q$ G protein, a *Limulus polyphemus* $G\alpha_q$ G protein, a *Patinopecten yessoensis* $G\alpha_q$ G protein, a *Loligo forbesi* $G\alpha_q$ G protein, a *Homarus americanus* $G\alpha_q$ G protein, a *Lymnaea stagnalis* $G\alpha_q$ G protein, a *Geodia cydonium* $G\alpha_q$ G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of

such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

In one embodiment of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention provides a process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using any of the aforementioned processes and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. The invention also provides a process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by any of the aforementioned processes or a novel structural and functional analog or homolog thereof.

GPCRs that can be used with the invention include, but are not limited to, neuropeptide FF receptors, e.g., human NPFF1 (ATCC Accession number 203605) and human NPFF2 (ATCC Accession number 203255). Plasmid pCDNA3.1-hNPFF1 and plasmid pCDNA3.1-hNPFF2b were deposited on January 21, 1999 and September 22, 1998, respectively, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession Nos. 203605 and 203255, respectively.

Further GPCRs that can be used with the invention include, but are not limited to, serotonin receptors, e.g., human 5HT1D (U.S. Patent No. 5,155,218, the disclosure of which is hereby incorporated by reference in its entirety into this application), rabbit 5HT1D (Harwood, G. et al., 1995), human 5HT7 (ATCC Accession number 75332), human 5HT1E (U.S. Patent No. 5,476,782, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT1F (U.S. Patent No. 5,360,735, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT5A (Plassat et al., 1992), human 5HT5B (Matthes et al., 1993), human 5HT1B (U.S. Patent No. 5,155,218, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT4 (U.S. Patent No. 5,766,879, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT6 (Kohen et al., 1996), and human 5HT1A (Kobilka et al., 1987). Plasmid pcEXV-5HT_{4B} was deposited on October 20, 1992 with the

American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of
 5 Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 75332.

Further GPCRs that can be used with the invention include, but are not limited to, dopamine receptors,
 10 e.g., human D1, human D2, human D3, and human D5 (U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application), and alpha adrenergic receptors, e.g., human α 1A adr, human α 2C adr, human
 15 α 2B adr, human α 2A adr (U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application), and human β 2 adr (Dixon et al., 1986).

Further GPCRs that can be used with the invention include, but are not limited to, galanin receptors, e.g., human GALR1 (Habert-Ortoli et al., 1994), rat GALR1 (Burgevin et al., 1995), human GALR2 (ATCC Accession No. 97851), rat GALR2 (ATCC Accession No.
 20 97426), human GALR3 (ATCC Accession No. 97827), and rat GALR3 (ATCC Accession No. 97826). Plasmids pEXJ-hGalR3 and pEXJ-rGALR3T were deposited on December 17, 1996, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia
 25 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession Nos. 97827 and 97826, respectively. Plasmids B039 and
 30 K985 were deposited on January 15, 1997 and January 24, 1996, respectively, with the American Type
 35

Culture Collection (ATCC), 10801 University Blvd.,
Manassas, Virginia 20110-2209, U.S.A. under the
provisions of the Budapest Treaty for the
International Recognition of the Deposit of
5 Microorganisms for the Purposes of Patent Procedure
and were accorded ATCC Accession Nos. 97851 and
97426, respectively.

Further GPCRs that can be used with the invention
10 include, but are not limited to, neuropeptide Y
receptors, e.g., human Y1 (Larhammar et al., 1992),
rat Y1 (Eva et al., 1990), human Y2 (U.S. Patent No.
5,545,549, the disclosure of which is hereby
15 incorporated by reference in its entirety into this
application), human Y4 (U.S. Patent No. 5,516,653,
the disclosure of which is hereby incorporated by
reference in its entirety into this application), rat
Y4 (ATCC Accession No. 75984), human Y5 (U.S. Patent
No. 5,602,024, the disclosure of which is hereby
20 incorporated by reference in its entirety into this
application), and rat Y5 (U.S. Patent No. 5,602,024,
the disclosure of which is hereby incorporated by
reference in its entirety into this application).
Plasmid pcEXV-rY4 was deposited on December 21, 1994
25 with the American Type Culture Collection (ATCC),
10801 University Blvd., Manassas, Virginia 20110-
2209, U.S.A. under the provisions of the Budapest
Treaty for the International Recognition of the
Deposit of Microorganisms for the Purposes of Patent
30 Procedure and were accorded ATCC Accession No. 75984.

Further GPCRs that can be used with the invention
include, but are not limited to, neurotensin
receptors, e.g., rat NTR1 (Tanaka et al., 1990);
35 glucagon-like peptide receptors, e.g., human GLP-1
(Dillon et al., 1993); kappa opioid receptors, e.g.,

human kappa (Mansson et al., 1994); and melanin
concentrating hormone receptors, e.g., human MCH
(ATCC Accession No. 203197). Plasmid pEXJ.HR-TL231
was deposited on September 17, 1998 with the American
5 Type Culture Collection (ATCC), 10801 University
Blvd., Manassas, Virginia 20110-2209, U.S.A. under
the provisions of the Budapest Treaty for the
International Recognition of the Deposit of
Microorganisms for the Purposes of Patent Procedure
10 and were accorded ATCC Accession No. 203197.

The invention will be better understood from the
Experimental Details which follow. However, one
skilled in the art will readily appreciate that the
15 specific methods and results discussed are merely
illustrative of the invention as described more fully
in the claims which follow thereafter.

Experimental Details

Materials and Methods

5 Cloning of the gene encoding *C. elegans* $G\alpha_q$

The gene for wild-type *C. elegans* $G\alpha_q$ was obtained by PCR amplification of a mixed stage *C. elegans* cDNA library (Stratagene, #937006) with the primers RP65 and RP66 (Table 1). The resulting product was cloned into the vector pcDNA 3.1 Zeo (Invitrogen) at the KpnI and XbaI sites. DNA sequence analysis demonstrated that the clone designated R48 was identical to that of the *C. elegans* $G\alpha_q$ gene deposited in Genbank (accession number AF003739).

15 Cloning of the gene encoding *D. melanogaster* $G\alpha_q$

The gene for wild-type *D. melanogaster* $G\alpha_q$ (isoform 3) was obtained by PCR amplification of *D. melanogaster* cDNA using primers RP203 and RP204 (Table 1). The resulting product was cloned into pcDNA3.1 (Invitrogen) at the KpnI and EcoRI sites. DNA sequence analysis demonstrated that the clone designated R129 encoded a protein identical to that of the *D. melanogaster* $G\alpha_{q3}$ gene deposited in Genbank (accession number P54400).

25 Cloning of genes encoding human $G\alpha_q$

The sequence of human $G\alpha_q$ was confirmed by automated sequence analysis. Except for the substitution of a single amino acid at position 171 (Ala \rightarrow Ser) in a highly non-conserved region of the protein, the deduced amino acid sequence is identical to that of Accession Number L76256. This sequence was used to generate the various human chimerae described throughout this application, except as noted in Table

9. A second human $G\alpha_q$ clone was obtained using standard PCR-based techniques that has a sequence identical to Genbank entry L76256. As expected, chimerae utilizing these two independently derived human $G\alpha_q$ sequences were found to be functionally indistinguishable in parallel assays (Table 9), using the dopamine D1 receptor as an example.

Construction of G protein cDNAs with chimeric 3' ends

Most of the chimeric G protein cDNAs were made by a PCR approach (Table 2). In each case, the designated primers were used to amplify the 3' end of the appropriate template to generate a chimeric PCR product. This product was then subcloned back into wild-type human, *D. melanogaster*, or *C. elegans* $G\alpha_q$, as appropriate, to generate a full-length chimeric gene. All PCR derived sequences were verified by sequence analysis. Two chimeras (Table 3) were constructed using the QuikChange site-directed mutagenesis kit (Stratagene, #200518). For these clones, the sequence of the entire coding region was verified. Examples of chimeric G proteins used in the present application are depicted in Figure 2.

TABLE 1. Primer sequences used in the preparation of chimeric G protein genes

PRIMER	SEQUENCE
MJ177	5' GAATATGATGGACCCCAGAGAGATG 3' (SEQ ID NO: 19)
MJ178	5' GATCCTCGAGTTAGCACAGTCCGATGTACTTCAGGTTC AACTGGAGGATGGT 3' (SEQ ID NO: 20)
MJ180	5' GATCCTCGAGTTAGTACAGTCCGCATCCCTTCAGGTTC ACTGGAGGATGGT 3' (SEQ ID NO: 21)
MJ193	5' GATCCTCGAGTTAGTAAAGCCCACATTCCTTCAGGTTC AACTGGAGGATGGT 3' (SEQ ID NO: 22)

MJ194	5' GATCCTCGAGTTAGAGCAGCTCGTATTGCTTCAGGTTCA ACTGGAGGATGGT 3' (SEQ ID NO: 23)
MJ197	5' GGAAAAAAGCGGCCGCTTAAACAGTCCGCAGTCC TTCAGGTTCAACTGGAGGATGGT 3' (SEQ ID NO: 24)
RP65	5' GGGGTACCGCCGCCATGGCCTGCTGTTTATCC 3' (SEQ ID NO: 25)
RP66	5' GCTCTAGATTACACCAAGTTGTACTCCTTCAGATT 3' (SEQ ID NO: 26)
RP80	5' CTCTCCGATCTCCGACGGCTG 3' (SEQ ID NO: 27)
RP83	5' TTCTACAGCATAATCTGAAGTATATCGGTTTGTGTTAATCT AGAGGGCCCGTTTAAACCCGCTG 3' (SEQ ID NO: 28)
RP84	5' CAGCGGGTTTAAACGGGGCCCTCTAGATTAACACAAACCGAT ATACTTCAGATTATGCTGTAGAA 3' (SEQ ID NO: 29)
RP85	5' CAGCATAATCTGAAGGAGTGTGGATTGTACTAATCTAGA GGGCCCCG 3' (SEQ ID NO: 30)
RP86	5' CGGGCCCTCTAGATTAGTACAATCCACACTCCTTCAG ATTATGCTG 3' (SEQ ID NO: 31)
RP116	5' GGAAAAAAGCGGCCGCTTAGAGCAGCTCGTATTGC CTCAGGTGCATCTGGAGGATGGTGTCTTGACGG 3' (SEQ ID NO: 32)
RP142	5' GCTCTAGATTAGAGCAGCTCGTATTGCCTCAGGTGCATCTG TAGAATTGTGTCTTTGACGGCG 3' (SEQ ID NO: 33)
RP168	5' GCTCTAGATTAACATAGCCCTATGTATTTTAGATTATTCTG TAGAATTGTGTCTTTGACGGCG 3' (SEQ ID NO: 34)
RP177	5' GCTCTAGATTAGAGCAGCTCGTATTGCCTCAGGTGCATACG TTGAATAATGTCACGACAGTCATTAAAAACACGCCGAATGT TTTCCGTATCAGTCGC 3' (SEQ ID NO: 35)

RP203	5' CGGGGTACCCCGGTTAGCATGGAGTGCTGTTTATCG 3' (SEQ ID NO: 42)
RP204	5' CCGGAATTCCGGTTAGACCAAATTATATTCCTTAAGGTTC 3' (SEQ ID NO: 43)
RP218	5' GAGCATCGATTACGAGACCGTTACC 3' (SEQ ID NO: 44)
RP219	5' CGGAATTCTTAGCACAGTCCGATGTACTTAAGGTTCGATTG CAGAATTGTGTC 3' (SEQ ID NO: 45)

TABLE 2. Primer pairs used to generate chimeric genes by PCR

CHIMERA	PCR TEMPLATE	PRIMERS
Human $G\alpha_{q/z5}$	$hG\alpha_q$	MJ177 / MJ178
Human $G\alpha_{q/12(5)}$	$hG\alpha_q$	MJ177 / MJ197
Human $G\alpha_{q/13(5)}$	$hG\alpha_q$	MJ177 / MJ193
Human $G\alpha_{q/o5}$	$hG\alpha_q$	MJ177 / MJ180
Human $G\alpha_{q/s5}$	$hG\alpha_q$	MJ177 / MJ194
Human $G\alpha_{q/s9}$	$hG\alpha_q$	MJ177 / RP116
<i>C.elegans</i> $G\alpha_{q/s9}$	R48	RP80 / RP142
<i>C. elegans</i> $G\alpha_{q/s21}$	R48	RP80 / RP177
<i>C. elegans</i> $G\alpha_{q/z9}$	R48	RP80 / RP168
<i>D. melanogaster</i> $G\alpha_{q/z}$	R129	RP218/ RP219

TABLE 3. Primer pairs used to generate chimeric genes using mutagenesis

CHIMERA	TEMPLATE	PRIMERS
<i>C.elegans</i> $G\alpha_{q/13(5)}$	R48	RP85 / RP86
<i>C.elegans</i> $G\alpha_{q/z5}$	R48	RP83 / RP84

General methods of transfecting cells

Methods of transfecting cells, e.g. mammalian cells, with such nucleic acid encoding a GPCR to obtain cells in which the GPCR is expressed on the surface of the cell are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.) The cells may be additionally transfected with nucleic acid encoding chimeric G proteins to obtain cells in which both the GPCR and the chimeric G proteins are expressed in the cell.

Such transfected cells may also be used to test compounds and screen compound libraries to obtain compounds which bind receptors as well as compounds which activate or inhibit activation of functional responses in such cells, and therefore are likely to do so in vivo. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

Host cells

A broad variety of host cells can be used to study heterologously expressed proteins. These cells include but are not limited to mammalian cell lines such as; Cos-7, CHO, LM(tk⁻), HEK293, etc.; insect cell lines such as; Sf9, Sf21, etc.; amphibian cells

such as *Xenopus* oocytes; assorted yeast strains; assorted bacterial cell strains; and others. Culture conditions for each of these cell types is specific and is known to those familiar with the art.

5

Transient expression

DNA encoding proteins to be studied can be transiently expressed in a variety of mammalian, insect, amphibian, yeast, bacterial and other cells lines by several transfection methods including but not limited to: calcium phosphate-mediated, DEAE-dextran mediated; liposomal-mediated, viral-mediated, electroporation-mediated, and microinjection delivery. Each of these methods may require optimization of assorted experimental parameters depending on the DNA, cell line, and the type of assay to be subsequently employed.

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Stable expression

Heterologous DNA can be stably incorporated into host cells, causing the cell to perpetually express a foreign protein. Methods for the delivery of the DNA into the cell are similar to those described above for transient expression but require the co-transfection of an ancillary gene to confer drug resistance on the targeted host cell. The ensuing drug resistance can be exploited to select and maintain cells that have taken up the DNA. An assortment of resistance genes are available including but not restricted to neomycin, kanamycin, and hygromycin.

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Mammalian cell tissue culture and transfection.

COS-7 cells were cultured in 225 cm² flasks in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine,

35

100 units/ml penicillin and 100 µg/ml streptomycin) at 37°C, 5% CO₂. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days.

5 GPCR and chimeric G protein cDNAs were transiently transfected into COS-7 cells in 150 cm² flasks by the DEAE-dextran method (Cullen, 1987), using a total of 20 µg of DNA/ ~ 7 x 10⁶ cells. For evaluating the function of a single chimeric G protein, the standard
10 cDNA transfection ratio was 1:1 (10 µg GPCR cDNA and 10 µg chimeric G protein cDNA). For evaluating the function of a mixture of chimeric G proteins, the standard cDNA transfection ratio was 8:1:1 (16 µg GPCR cDNA, 2 µg Gα_{q/z} cDNA, 2 µg Gα_{q/s} cDNA).

15

Membrane preparations

Cell membranes expressing the heterologously expressed proteins of this invention are useful for certain types of assays including but not restricted
20 to ligand binding assays, GTPγS binding assays, and others. The specifics of preparing such cell membranes may in some cases be determined by the nature of the ensuing assay but typically involve harvesting whole cells and disrupting the cell pellet by sonication in ice cold buffer (e.g. 20 mM Tris-
25 HCl, 5 mM EDTA, pH 7.4). The resulting crude cell lysate is cleared of cell debris by low speed centrifugation at 200xg for 5 min at 4°C. The cleared supernatant is then centrifuged at 40,000xg
30 for 20 min at 4°C, and the resulting membrane pellet is washed by suspending in ice cold buffer and repeating the high speed centrifugation step. The final washed membrane pellet is resuspended in assay buffer. Protein concentrations are determined by the
35 method of Bradford (1976) using bovine serum albumin

as a standard. The membranes may be used immediately or frozen for later use.

Generation of baculovirus

5 The coding region of DNA encoding the human receptor and the chimeric G protein disclosed herein may be separately subcloned into pBlueBacIII into existing restriction sites or sites engineered into sequences 5' and 3' to the coding region of the polypeptides.

10 To generate baculovirus, 0.5 µg of viral DNA (BaculoGold) and 3 µg of DNA construct encoding a polypeptide may be co-transfected into 2×10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells are then incubated for 5 days at 27°C.

20 The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

Binding assays

25 Labeled ligands are placed in contact with either membrane preparations or intact cells expressing the chimeric G protein and receptor of interest in multi-well microtiter plates, together with unlabeled compounds, and binding buffer. Binding reaction mixtures are incubated for times and temperatures determined to be optimal in separate equilibrium binding assays. The reaction is stopped by filtration through GF/B filters, using a cell harvester, or by directly measuring the bound ligand.

35 If the ligand was labeled with a radioactive isotope

such as ^3H , ^{14}C , ^{125}I , ^{35}S , ^{32}P , ^{33}P , etc., the bound ligand may be detected by using liquid scintillation counting, scintillation proximity, or any other method of detection for radioactive isotopes. If the ligand was labeled with a fluorescent compound, the bound labeled ligand may be measured by methods such as, but not restricted to, fluorescence intensity, time resolved fluorescence, fluorescence polarization, fluorescence transfer, or fluorescence correlation spectroscopy. In this manner, agonist or antagonist compounds that bind to the receptor may be identified as they inhibit the binding of the labeled ligand to the membrane protein or intact cells expressing the said receptor. Non-specific binding is defined as the amount of labeled ligand remaining after incubation of membrane protein in the presence of a high concentration (e.g., 100-1000 X K_D) of unlabeled ligand. In equilibrium saturation binding assays membrane preparations or intact cells transfected with the chimeric G protein and GPCR are incubated in the presence of increasing concentrations of the labeled compound to determine the binding affinity of the labeled ligand. The binding affinities of unlabeled compounds may be determined in equilibrium competition binding assays, using a fixed concentration of labeled compound in the presence of varying concentrations of the displacing ligands.

Functional assays

Cells expressing the chimeric G protein DNA of this invention and a GPCR may be used to screen for ligands to the GPCR using functional assays. Once a ligand is identified, the same assays may be used to identify agonists or antagonists of the GPCR that may be employed for a variety of therapeutic purposes.

It is well known to those in the art that the over-expression of a G protein-coupled receptor can result in the constitutive activation of intracellular signaling pathways. In the same manner, over-expression of an orphan receptor and a chimeric G protein in any cell line as described above, can result in the activation of the functional responses described below, and any of the assays herein described can be used to screen for both agonist and antagonist ligands of the orphan receptor.

A wide spectrum of assays can be employed to screen for the presence of orphan receptor ligands or to identify agonists or antagonists of a characterized GPCR. These assays range from traditional measurements of total inositol phosphate accumulation, cAMP levels, intracellular calcium mobilization, and potassium currents, for example; to systems measuring these same second messengers, but which have been modified or adapted to be of higher throughput, more generic, and more sensitive; to cell based assays reporting more general cellular events resulting from receptor activation such as metabolic changes, differentiation, cell division/proliferation. Description of several such assays follow.

Cyclic AMP (cAMP) assay

Elevation of intracellular Ca^{++} can modulate the activity of adenylyl cyclases via Ca^{++} -dependent calmodulin (Sunahara et al., 1996). The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing a GPCR and chimeric G protein. Cells are plated in 96-well plates or other vessels and preincubated in a buffer such as HEPES buffered saline (NaCl (150 mM),

CaCl₂ (1 mM), KCl (5 mM), glucose (10 mM)) supplemented with a phosphodiesterase inhibitor such as 5mM theophylline, with or without protease inhibitor cocktail for 20 min at 37°C, in 5% CO₂. A typical inhibitor cocktail contains 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon. Test compounds are added with or without 10 mM forskolin and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl or other methods. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution is measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software. Specific modifications may be performed to optimize the assay for the GPCR or to alter the detection method of cAMP.

Arachidonic acid release assays

Cells expressing a GPCR and chimeric G protein are seeded into 96 well plates or other vessels and grown for 3 days in medium with supplements. ³H-arachidonic acid (specific activity = 0.75 µCi/ml) is delivered as a 100 µL aliquot to each well and samples are incubated at 37° C, 5% CO₂ for 18 hours. The labeled cells are washed three times with medium. The wells are then filled with medium and the assay is initiated with the addition of test compounds or buffer in a total volume of 250 µL. Cells are incubated for 30 min at 37°C, 5% CO₂. Supernatants are transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples are then dissolved and resuspended in 25 µL distilled water. Scintillant (300 µL) is added to each well

and samples are counted for ^3H in a Trilux plate reader. Data are analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

5

Intracellular calcium mobilization assay

Twenty four hours after transient transfection, COS-7 cells were seeded into 96-well black wall microtiter plates coated with poly-D-lysine for assay the following day. Just prior to assay, culture medium was aspirated and cells were dye-loaded with 4 μM Fluo-3/ 0.01% pluronic acid in assay buffer composed of Hank's Balanced Salt Solution (138 mM NaCl, 5 mM KCl, 1.3 mM CaCl_2 , 0.5 mM MgCl_2 , 0.4 mM MgSO_4 , 0.3 mM KH_2PO_4 , 0.3 mM Na_2HPO_4 , 5.6 mM glucose) plus 20 mM HEPES (pH 7.4), 0.1% BSA and 2.5 mM probenidol (100 μl /well) for 1 hour in 5% CO_2 at 37 $^\circ\text{C}$. After excess dye was discarded, cells were washed in assay buffer and layered with a final volume equal to 100 μl /well. Basal fluorescence was monitored in a fluorometric imaging plate reader (FLIPRTM, Molecular Devices) with an excitation wavelength of 488 nm and an emission range of 500 to 560 nm. Laser excitation energy was adjusted so that basal fluorescence readings were approximately 10,000 relative fluorescent units. Cells were stimulated with agonists diluted in assay buffer (50 μl), and relative fluorescent units were measured at defined intervals (exposure = 0.4 sec) over a 3 min period at room temperature. Maximum change in fluorescence was calculated for each well. Concentration-response curves derived from the maximum change in fluorescence were analyzed by nonlinear regression (Hill equation).

Alternatively, intracellular free calcium concentration may be measured by microspectrofluorimetry using the fluorescent indicator dye Fura-2/AM (Bush et al, 1991). Cells expressing the receptor and chimeric G protein are seeded onto a 35 mm culture dish containing a glass coverslip insert and allowed to adhere overnight. Cells are then washed with HBS and loaded with 100 μ L of Fura-2/AM (10 μ M) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nm with excitation wavelengths alternating between 340 nm and 380 nm. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

Alternative calcium-sensitive indicators may be used. Preferred examples of these are: aequorin, Fluo-3, Fluo-4, Fluo-5, Calcium Green-1, Oregon Green, and 488 BAPTA. After activation of the receptors with agonist ligands the emission elicited by the change of intracellular calcium concentration can be measured by a luminometer or a fluorescence imager; a preferred example of this is the fluorescence imager plate reader (FLIPR™) as described above. Antagonist ligands are identified by the inhibition of the signal elicited by agonist ligands.

Inositol phosphate assay

Receptor mediated activation of the inositol phosphate (IP) second messenger pathways may be assessed by radiometric or other measurement of IP products. For example, in a 96 well microplate format

assay, cells are plated at a density of 70,000 cells per well and allowed to incubate for 24 hours. The cells are then labeled with 0.5 μCi [^3H]myo-inositol overnight at 37°C, 5% CO_2 . Immediately before the

5 assay, the medium is removed and replaced with 90 μL of PBS containing 10 mM LiCl. The plates are then incubated for 15 min at 37°C, 5% CO_2 . Following the incubation, the cells are challenged with agonist (10 μl /well; 10x concentration) for 30 min at 37°C, 5%

10 CO_2 . The challenge is terminated by the addition of 100 μL of 50% v/v trichloroacetic acid, followed by incubation at 4°C for greater than 30 minutes. Total IPs are isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the

15 wells are transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared adding 100 μL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates are

20 placed on a vacuum manifold to wash or elute the resin bed. Each well is first washed 2 times with 200 μl of 5 mM myo-inositol. Total [^3H]inositol phosphates are eluted with 75 μl of 1.2 M ammonium formate/0.1 M formic acid solution into 96-well

25 plates. 200 μL of scintillation cocktail is added to each well and the radioactivity is determined by liquid scintillation counting.

GTP γ S binding assay

30 Membranes from cells expressing a GPCR and a chimeric G protein are suspended in assay buffer (e.g., 50 mM Tris, 100 mM NaCl, 5 mM MgCl_2 , 10 μM GDP, pH 7.4) with or without protease inhibitors (e.g., 0.1% bacitracin). Membranes are incubated on ice for 20

35 minutes, transferred to a 96-well Millipore

microtiter GF/C filter plate and mixed with GTP γ ³⁵S (e.g., 250,000 cpm/sample, specific activity ~1000 Ci/mmol) plus or minus unlabeled GTP γ S (final concentration = 100 μ M). The final membrane protein concentration is approximately 20 μ g/ml. Samples are incubated in the presence or absence of test compounds for 30 minutes at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold (4⁰C) assay buffer. Samples collected in the filter plate are treated with scintillant and counted for ³⁵S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an expression system resulting in high levels of expression of the receptor and/or expressing G proteins having high turnover rates (for the exchange of GDP for GTP). GTP γ S assays are well-known to those skilled in the art, and it is contemplated that variations on the method described above, such as are described by Tian et al. (1994) or Lazareno and Birdsall (1993), may be used.

MAP kinase assay

MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (such as Gq /G11 -coupled) produce

diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

5 MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated (inactive) or phosphorylated (active). The phosphorylated protein has a slower mobility in SDS-
10 PAGE and can therefore be compared with the unstimulated protein using Western blotting. Alternatively, antibodies specific for the phosphorylated protein are available (New England Biolabs) which can be used to detect an increase in
15 the phosphorylated kinase. In either method, cells are stimulated with the test compound and then extracted with Laemmli buffer. The soluble fraction is applied to an SDS-PAGE gel and proteins are transferred electrophoretically to nitrocellulose or
20 Immobilon. Immunoreactive bands are detected by standard Western blotting technique. Visible or chemiluminescent signals are recorded on film and may be quantified by densitometry.

25 Another approach is based on evaluation of the MAP kinase activity via a phosphorylation assay. Cells are stimulated with the test compound and a soluble extract is prepared. The extract is incubated at 30°C for 10 min with gamma-³²P-ATP, an ATP
30 regenerating system, and a specific substrate for MAP kinase such as phosphorylated heat and acid stable protein regulated by insulin, or PHAS-I. The reaction is terminated by the addition of H₃PO₄ and samples are transferred to ice. An aliquot is
35 spotted onto Whatman P81 chromatography paper, which retains the phosphorylated protein. The

chromatography paper is washed and counted for ^{32}P in a liquid scintillation counter. Alternatively, the cell extract is incubated with gamma- ^{32}P -ATP, an ATP regenerating system, and biotinylated myelin basic protein bound by streptavidin to a filter support. The myelin basic protein is a substrate for activated MAP kinase. The phosphorylation reaction is carried out for 10 min at 30°C . The extract can then be aspirated through the filter, which retains the phosphorylated myelin basic protein. The filter is washed and counted for ^{32}P by liquid scintillation counting.

Cell proliferation assay

Receptor activation of a GPCR may lead to a mitogenic or proliferative response which can be monitored via ^3H -thymidine uptake. When cultured cells are incubated with ^3H -thymidine, the thymidine translocates into the nuclei where it is phosphorylated to thymidine triphosphate. The nucleotide triphosphate is then incorporated into the cellular DNA at a rate that is proportional to the rate of cell growth. Typically, cells are grown in culture for 1-3 days. Cells are forced into quiescence by the removal of serum for 24 hrs. A mitogenic agent is then added to the media. Twenty-four hrs later, the cells are incubated with ^3H -thymidine at specific activities ranging from 1 to 10 $\mu\text{Ci/ml}$ for 2-6 hrs. Harvesting procedures may involve trypsinization and trapping of cells by filtration over GF/C filters with or without a prior incubation in TCA to extract soluble thymidine. The filters are processed with scintillant and counted for ^3H by liquid scintillation counting. Alternatively, adherent cells are fixed in MeOH or TCA, washed in water, and solubilized in 0.05%

deoxycholate/0.1 N NaOH. The soluble extract is transferred to scintillation vials and counted for ^3H by liquid scintillation counting.

5 Alternatively, cell proliferation can be assayed by measuring the expression of an endogenous or heterologous gene product, expressed by the cell line used to transfect the GPCR, which can be detected by methods such as, but not limited to, fluorescence
10 intensity, enzymatic activity, immunoreactivity, DNA hybridization, polymerase chain reaction, etc.

Reporter gene assays

The chimeric $\text{G}\alpha$ subunits described in this application can be used in conjunction with any
15 number of $\text{G}\alpha_q$ -linked transcriptional assays to include GPCRs that do not normally use $\text{G}\alpha_q$ as their native signaling pathway. This application could include, but is not limited to, the use of $\text{G}\alpha$
20 chimeras to link activation of any GPCR to a fluorescent signal generated via a reporter enzyme such as β -lactamase placed under the transcriptional regulation of NFAT, SRE, CRE, AP-1, TRE IRE or other specific DNA regulatory elements or promoters
25 (Naylor, 1999).

Methods for recording currents in *Xenopus* oocytes

Oocytes were harvested from *Xenopus laevis* and injected with mRNA transcripts as previously
30 described (Quick and Lester, 1994; Smith et al., 1997). Receptor and chimeric G protein α subunit RNA transcripts were synthesized using the T7 polymerase ("Message Machine," Ambion) from linearized plasmids or PCR products containing the complete coding region
35 of the genes. Oocytes were injected with 5-25 ng

synthetic receptor RNA and incubated for 3-8 days at 17 degrees. Three to eight hours prior to recording, oocytes were injected with 500 pg chimeric G α subunit mRNA. Dual electrode voltage clamp (Axon Instruments Inc.) was performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes were voltage clamped at a holding potential of -80 mV. During recordings, oocytes were bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES, pH 7.5 (ND96). Drugs were applied by local perfusion from a 10 μ l glass capillary tube fixed at a distance of 0.5 mm from the oocyte. Experiments were carried out at room temperature. All values are expressed as mean \pm standard error of the mean.

Beta-gamma-dependent signaling

Beta-gamma sub-units released from G α_q may interact with a variety of effectors, including phospholipase C beta, adenylate cyclase II and IV, ion channels (Kir 3.x family of K⁺ channels, calcium channels), Ras and PI-3-gamma. Each of these may be monitored by specific read-outs known to those skilled in the art.

Expression cloning

The expression cloning strategy is a well-known method utilized to clone mammalian G protein-coupled receptors (Kluxen et al., 1992; Kiefer et al, 1992; Julius et al., 1988; US 5,545,549 and US 5,602,024, the disclosures of which are hereby incorporated by reference in their entireties into this application). A chimeric G protein of this invention may be utilized in expression cloning to facilitate identification of clones which encode mammalian G protein-coupled receptors. Cells, expressing the DNA

encoding numerous independent clones, may be
transfected with and express DNA encoding a chimeric
G protein of this invention. The presence of the
chimeric G protein in the cells may facilitate ligand
5 activation of or binding to a mammalian G protein-
coupled receptor encoded by one of the independent
clones which may be subsequently isolated.

Results and Discussion

Expression of *C. elegans* chimera in *Xenopus* oocytes

The chimeric $G\alpha$ subunit consisting of $cG\alpha_{q/z5}$, wherein
 5 the C-terminal final 5 amino acids of $cG\alpha_q$ are
 replaced with those of $hG\alpha_z$ (Figure 2), was initially
 tested for expression and functional activity in
Xenopus oocytes. Co-expression of $cG\alpha_{q/z5}$ with the
 NPFF1 receptor resulted in the appearance of large
 10 amplitude Cl^- currents following application of 1 μM
 NPFF (1258 \pm 159 nA, $n = 12$). The currents
 stimulated by NPFF in oocytes expressing NPFF1 and
 $cG\alpha_{q/z5}$ were most likely mediated by the endogenous
 calcium-activated Cl^- channel (Gunderson et al.,
 15 1983), because they were blocked in oocytes injected
 with 50 nl of 10 mM EGTA (Figure 3). Chloride
 currents were also not observed from control oocytes
 expressing NPFF1 but lacking $cG\alpha_{q/z5}$ ($n=15$). In
 oocytes expressing NPFF1 and the human version of
 20 $G\alpha_{q/z5}$, response amplitudes (358 \pm 67, $n = 32$) were
 about one third of those in oocytes expressing the *C.*
elegans version of this chimera. Similar results were
 obtained with four additional GPCRs, GALR1, Y1,
 NPFF2, and 5HT1D, that are known to couple to either
 25 $G\alpha_i$ or $G\alpha_o$ (Table 4; Watling, 1998). The increase in
 response was 2-3 fold over currents recorded from
 oocytes expressing the human version of the chimera.
 The exception to this trend was coupling to the Y5
 receptor, which was actually reduced with $cG\alpha_{q/z5}$.
 30 Extending the length of the $G\alpha_z$ portion of the C-
 terminal tail of $cG\alpha_q$ to 9 amino acids ($cG\alpha_{q/z9}$) did
 not further improve the amplitude of responses as
 compared to $cG\alpha_{q/z5}$ (Table 4).

TABLE 4. Response amplitudes (nA) in oocytes expressing various GPCRs and chimeric G proteins. Values are mean \pm s.e.m (number of oocytes).

RECEPTOR	CHIMERA		
	hG $\alpha_{q/z5}$	cG $\alpha_{q/z5}$	cG $\alpha_{q/z9}$
Rabbit 5HT1D	90 \pm 41 (14)	150 \pm 105 (8)	34 \pm 12 (9)
Rat GALR1	31 \pm 16 (22)	91 \pm 38 (15)	Not tested
Human NPFF1	358 \pm 67 (32)	1258 \pm 159 (12)	1449 \pm 398 (5)
Human NPFF2	528 \pm 99 (18)	1121 \pm 261 (13)	Not tested
Rat Y1	841 \pm 204 (19)	1549 \pm 168 (13)	300 \pm 177 (8)
Rat Y5	82 \pm 43 (7)	0 \pm 2 (8)	65 \pm 34 (6)

5

Expression of chimeras in mammalian cells

To evaluate the utility of cG $\alpha_{q/z5}$ in mammalian cells, COS-7 cells were transiently transfected with either hG $\alpha_{q/z5}$ or cG $\alpha_{q/z5}$ plus a GPCR. In one example, cells transfected either with hG $\alpha_{q/z5}$ or cG $\alpha_{q/z5}$ plus the human D1 receptor, which is thought to be G α_s - and G $\alpha_{1/o}$ -coupling (Sidhu et al., 1991), were stimulated with dopamine at concentrations up to 100 μ M and monitored for calcium mobilization (Figure 4). Whereas an agonist-induced response was undetectable with hG $\alpha_{q/z5}$ (n = 2), the cG $\alpha_{q/z5}$ construct supported an average maximum dopamine-stimulated signal of 12,120 relative fluorescence units (n = 2).

15

The data for human D1 clearly demonstrate that the probability of GPCR signal detection in mammalian cells can be enhanced by the use of a chimeric construct containing an invertebrate $G\alpha_q$ backbone (*C. elegans* $G\alpha_q$ in this case). To determine whether this effect extends to a broad range of GPCRs, c $G\alpha_{q/z5}$ was co-transfected into COS-7 cells with a panel of 36 different GPCRs, including $G\alpha_{i/o}$ -, $G\alpha_s$ -, and $G\alpha_q$ -coupling receptors. Seventy eight percent (28/36) of the receptors generated positive signals (defined as > 500 fluorescence units) with c $G\alpha_{q/z5}$, compared to only 58% with h $G\alpha_{q/z5}$ (Table 5). Extending the $G\alpha_z$ tail length from 5 to 9 amino acids did not significantly change the detection rate (29/36 positive responses > 500 fluorescence units) but there was a trend, particularly among the most responsive receptors, toward a decreased maximal response. From these data, we can conclude that an invertebrate-based $G\alpha_{q/z}$ construct is optimal for detecting a broad range of GPCR, and we can identify c $G\alpha_{q/z5}$ as a preferred design.

TABLE 5. $G\alpha_{q/z5}$ chimeras and GPCR in COS-7 cells: agonist-induced response. Transfected cells were monitored for calcium mobilization in the FLIPR™ using the calcium sensitive dye Fluo-3. Maximum agonist concentrations were 100 μ M for non-peptide ligands or 10 μ M for peptide ligands, except for neurotensin (1 μ M). Fluorescence data represent the mean from 2 or more experiments. h = human; m = mouse; r = rat; adr = adrenergic; DA = dopamine; GLP-1 = glucagon-like peptide; 5HT = serotonin; GAL = galanin; NE = norepinephrine; MCH = melanin-concentrating hormone; NPY = neuropeptide Y; PP =

pancreatic polypeptide; NPFF = neuropeptide FF; and
NT = neurotensin

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)		
				$hG\alpha_{q/z5}$	$cG\alpha_{q/z5}$	$cG\alpha_{q/z9}$
1	h D3	$G\alpha_{1/o}$	DA	0	0	121
2	h GLP-1	$G\alpha_s$	GLP-1	287	131	135
3	h 5HT7	$G\alpha_s$	5HT	90	166	129
4	h 5HT1E	$G\alpha_{1/o}$	5HT	202	229	238
5	h 5HT1F	$G\alpha_{i/o}$	5HT	0	243	384
6	m 5HT5B	$G\alpha_{1/o}$	5HT	251	265	443
7	m 5HT5A	$G\alpha_{i/o}$	5HT	0	351	270
8	h 5HT1D	$G\alpha_{1/o}$	5HT	316	414	504
9	h D5	$G\alpha_s, G\alpha_{i/o}$	DA	782	657	797
10	h 5HT1B	$G\alpha_{1/o}$	5HT	405	929	1217
11	h 5HT4	$G\alpha_s, G\alpha_q$	5HT	2161	1011	1696
12	h 5HT6	$G\alpha_s$	5HT	210	1289	2287
13	h GALR3	$G\alpha_{i/o}$	GAL	804	1523	2050
14	h β_2 adr	$G\alpha_s, G\alpha_{i/o}$	NE	128	1842	1697
15	h 5HT1A	$G\alpha_{i/o}$	5HT	478	1997	3139
16	r GALR3	$G\alpha_{1/o}$	GAL	2796	2298	2971
17	h MCH	$G\alpha_q$	MCH	783	2699	3332
18	r GALR1	$G\alpha_{1/o}$	GAL	82	3086	5947
19	r Y4	$G\alpha_{1/o}$	PP	4388	3662	2583
20	h α_2C adr	$G\alpha_{i/o}$	NE	6106	4143	3874
21	r GALR2	$G\alpha_q$	GAL	4862	4198	4470
22	h α_2B adr	$G\alpha_{i/o}$	NE	4515	4983	5138
23	h Y5	$G\alpha_{i/o}$	NPY	6407	5314	6680
24	h GALR2	$G\alpha_q$	GAL	5992	5470	4899
25	h kappa	$G\alpha_{1/o}$	U-69593	7864	5975	3472

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)		
				hG $\alpha_{q/z5}$	cG $\alpha_{q/z5}$	cG $\alpha_{q/z9}$
26	h NPFF1	G $\alpha_{i/o}$	NPFF	4717	6593	2966
27	h NPFF2	G $\alpha_{i/o}$	NPFF	19960	7566	4578
28	h $\alpha 2A$ adr	G $\alpha_{1/o}$	NE	10933	7575	3040
29	h D2	G $\alpha_{1/o}$	DA	15579	7615	4305
30	h GALR1	G $\alpha_{1/o}$	GAL	4061	7648	8489
31	h Y2	G $\alpha_{1/o}$	NPY	10908	7708	5387
32	h Y1	G $\alpha_{i/o}$	NPY	1879	7722	6728
33	h Y4	G $\alpha_{1/o}$	PP	9966	9422	7397
34	h $\alpha 1A$ adr	G α_q	NE	14167	9816	6597
35	h D1	G α_s , G $\alpha_{i/o}$	DA	0	12120	13099
36	r NTR1	G α_q	NT	11171	14476	6111

G $\alpha_{q/s}$ Chimeras

To identify additional uses for an invertebrate-based G α_q construct, modifications were made to the backbone and C-terminus of another type of chimera, G $\alpha_{q/s}$. Initially, the function of hG $\alpha_{q/s5}$ was compared with that of hG $\alpha_{q/s9}$. In one example, either construct was co-transfected into COS-7 cells with the human D1 receptor, which is typically G α_s - or G $\alpha_{1/o}$ -coupling (Sidhu et al., 1991). Transfected cells were stimulated with dopamine at concentrations up to 100 μ M and monitored for calcium mobilization. The average maximal agonist-induced response ranged from undetectable with hG $\alpha_{q/s5}$ (n = 2) to 5692 relative fluorescent units with hG $\alpha_{q/s9}$ (n = 4). The positive effect of increasing the G α_s tail length contrasts with data for *C. elegans* G $\alpha_{q/z}$ -type chimeras and has not been described previously (Conklin et

al., 1993, 1996). To further enhance signal detection, the human $G\alpha_q$ backbone was replaced with the corresponding sequence from *C. elegans* $G\alpha_q$. The modified construct, $cG\alpha_{q/s9}$, was co-transfected into
 5 COS-7 cells together with the human D1 receptor, and transfected cells were stimulated with dopamine at concentrations up to 100 μ M. The average maximal dopamine-stimulated fluorescent signal with $cG\alpha_{q/s9}$ was 8692 fluorescent units ($n = 4$), a 1.5-fold increase
 10 over the response with $hG\alpha_{q/s9}$. To test the general utility of $cG\alpha_{q/s9}$ for detection of $G\alpha_s$ -coupling receptors, this construct was co-transfected into COS-7 with a panel of 7 such GPCR. When cells were stimulated with appropriate agonists, 6/7 = 81% of
 15 the $G\alpha_s$ -coupling receptors generated positive responses (> 500 fluorescence units). Further extension of the C-terminal $G\alpha_s$ tail to 21 amino acids ($cG\alpha_{q/s21}$) yielded similar results overall, both in terms of detection rate and maximal response
 20 (Table 6).

TABLE 6. $G\alpha_{q/s}$ chimeras and G_s -coupled receptors in COS-7 cells: maximum agonist response. Transfected cells were monitored for calcium mobilization in the
 25 FLIPRTM using the calcium sensitive dye Fluo-3. Maximal agonist concentration was 100 μ M for non-peptide ligands or 10 μ M for GLP-1 (7-36) amide. Fluorescence data represent the mean from 2 or more experiments. h = human, adr = adrenergic, DA =
 30 dopamine; GLP-1 = glucagon-like peptide; 5HT = serotonin; NE = norepinephrine

#	RECEPTOR	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)			
			hG $\alpha_{q/s5}$	HG $\alpha_{q/s9}$	cG $\alpha_{q/s9}$	cG $\alpha_{q/s21}$
1	h GLP-1	GLP-1	189	4198	2461	3120
2	h 5HT7	5HT	0	0	387	206
3	h D5	DA	0	745	1870	3385
4	h 5HT4	5HT	1709	2309	1701	1731
5	h 5HT6	5HT	98	999	1639	1009
6	h β_2 adr	NE	43	1439	3106	3513
7	h D1	DA	0	5692	8692	9433

That the *C. elegans* backbone provides a signaling advantage when incorporated into either G $\alpha_{q/z}$ -type or G $\alpha_{q/s}$ -type chimeras suggests a novel and general method for designing effective chimeric constructs. In yet another example, human G $\alpha_{q/13(5)}$ was compared with *C. elegans* G $\alpha_{q/13(5)}$ using COS-7 cells transfected with the rat GALR3 receptor. The maximum signal produced by porcine galanin was 2084 relative fluorescent units with human G $\alpha_{q/13(5)}$ (n = 4), compared to 2564 fluorescent units with *C. elegans* G $\alpha_{q/13(5)}$ (n = 4). These data extend the range of possible uses for a *C. elegans* backbone in a G α_q chimeric construct.

Multiple chimerae strategies

Application of this technology to a high throughput screening paradigm (such as orphan receptor screening or expression cloning) requires that a maximal number of chimera-dependent receptors (G $\alpha_{i/o}$ and G α_s -coupling) can function under the same conditions as chimera-independent receptors (G α_q -coupled). One strategy, described above, is to use a single extremely promiscuous construct such as cG $\alpha_{q/z5}$.

Another strategy is to combine multiple chimeras in a transfection mixture. Ideally, the mixture should be reduced to its essential components, both in terms of individual chimera and corresponding cDNA or mRNA. A reductionist approach has several advantages: 1) it increases the allowance for cDNA or mRNA encoding the GPCR of interest; 2) it reduces potential competition for protein translation; and 3) it reduces the risk for dominant negative suppression of Gq-coupled receptor function. A simple and effective combination could be formed with a cG $\alpha_{q/z}$ -type chimera and a cG $\alpha_{q/s}$ -type chimera. In one example, a transfection mixture containing 2 μ g cG $\alpha_{q/z9}$ cDNA, 2 μ g cG $\alpha_{q/s9}$ cDNA, and 16 μ g GPCR cDNA was transfected into COS-7 cells for subsequent monitoring of calcium mobilization. Out of 36 receptors tested, 28 receptors = 78% were detectable upon agonist stimulation with maximal signals > 500 fluorescence units (Table 7). The detection rate was identical to that obtained previously with cG $\alpha_{q/z5}$ or cG $\alpha_{q/z9}$ alone, except that the two chimerae together favored detection of the G α_s -coupling receptor, human GLP-1. The use of multiple chimerae therefore represents an alternative method for screening various receptor types (G $\alpha_{i/Go}$ -, G α_s , and G α_q -coupled) in a single assay format.

TABLE 7. Chimerae cG $\alpha_{q/z9}$ and cG $\alpha_{q/s9}$ and GPCRs in COS-7 cells: agonist-induced responses. Two μ g cG $\alpha_{q/z9}$, 2 μ g cG $\alpha_{q/s9}$, and 16 μ g GPCR cDNA were transfected into COS-7 cells. Transfected cells were monitored for calcium mobilization in the FLIPRTM using the calcium sensitive dye Fluo-3. Maximum agonist concentrations were 100 μ M for non-peptide ligands or 10 μ M for peptide ligands, except for neurotensin (1 μ M).

Fluorescence data represent the mean from 2 or more experiments. h = human; m = mouse; r = rat; adr = adrenergic; DA = dopamine; GLP-1 = glucagon-like peptide; 5HT = serotonin; GAL = galanin; NE = norepinephrine; MCH = melanin-concentrating hormone; NPY = neuropeptide Y; PP = pancreatic polypeptide; NPFF = neuropeptide FF; and NT = neurotensin

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)
				$cG\alpha_{q/29} + cG\alpha_{q/s9}$
1	h D3	$G\alpha_{i/o}$	DA	208
2	h GLP-1	$G\alpha_s$	GLP-1	794
3	h 5HT7	$G\alpha_s$	5HT	292
4	h 5HT1E	$G\alpha_{1/o}$	5HT	2
5	h 5HT1F	$G\alpha_{i/o}$	5HT	247
6	m 5HT5B	$G\alpha_{i/o}$	5HT	0
7	m 5HT5A	$G\alpha_{i/o}$	5HT	45
8	h 5HT1D	$G\alpha_{1/o}$	5HT	433
9	h D5	$G\alpha_s, G\alpha_{1/o}$	DA	1172
10	h 5HT1B	$G\alpha_{1/o}$	5HT	190
11	h 5HT4	$G\alpha_s, G\alpha_q$	5HT	2345
12	h 5HT6	$G\alpha_s$	5HT	1598
13	h GALR3	$G\alpha_{1/o}$	GAL	853
14	h β_2 adr	$G\alpha_s, G\alpha_{i/o}$	NE	2346
15	h 5HT1A	$G\alpha_{1/o}$	5HT	2161
16	r GALR3	$G\alpha_{1/o}$	GAL	1402
17	h MCH	$G\alpha_q$	MCH	4808
18	r GALR1	$G\alpha_{1/o}$	GAL	1544
19	r Y4	$G\alpha_{1/o}$	PP	1015
20	h α_2C adr	$G\alpha_{i/o}$	NE	2341

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)
				$cG\alpha_{q/z9} + cG\alpha_{q/s9}$
21	r GALR2	$G\alpha_q$	GAL	2665
22	h $\alpha 2B$ adr	$G\alpha_{i/o}$	NE	4855
23	h Y5	$G\alpha_{1/o}$	NPY	982
24	h GALR2	$G\alpha_q$	GAL	4630
25	h kappa	$G\alpha_{i/o}$	U-69593	3529
26	h NPFF1	$G\alpha_{i/o}$	NPFF	793
27	h NPFF2	$G\alpha_{i/o}$	NPFF	1582
28	h $\alpha 2A$ adr	$G\alpha_{1/o}$	NE	5284
29	h D2	$G\alpha_{1/o}$	DA	5549
30	h GALR1	$G\alpha_{1/o}$	GAL	8097
31	h Y2	$G\alpha_{1/o}$	NPY	3329
32	h Y1	$G\alpha_{i/o}$	NPY	2333
33	h Y4	$G\alpha_{i/o}$	PP	4133
34	h $\alpha 1A$ adr	$G\alpha_q$	NE	7585
35	h D1	$G\alpha_s, G\alpha_{i/o}$	DA	13516
36	r NTR1	$G\alpha_q$	NT	4264

Summary of the results

5 This work describes a functional assay with which various types and large numbers of GPCRs can be detected. The method is based on the premise that $G\alpha$ proteins are derived from a common ancestor, and that the further a $G\alpha$ protein is evolutionarily from the ancestral sequence, the more likely it is to contain motifs which restrict interactions to a subset of GPCRs. Conversely, sequences from more primitive organisms such as invertebrates may lack the restrictive motifs. Focusing specifically on $G\alpha_q$, we

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performed an amino acid sequence alignment of all known protein structures and identified distinct motifs, which differentiate vertebrate from invertebrate species (Figure 5). For example, invertebrates lack the 6 amino acid N-terminal extension proposed to restrict GPCR interaction (Kostenis et al., 1998), and also contain Glu¹⁸-Lys¹⁹ instead of the vertebrate Ala¹⁸-Arg¹⁹ in a region of G α_q associated with receptor recognition (Lambright et al., 1996). These structural differences led us to speculate that an invertebrate G α_q backbone might function differently in a G α_q chimeric construct than would a vertebrate homologue, and that this difference might be expressed as an increase in GPCR/chimera promiscuity.

This hypothesis was tested using the invertebrate *C. elegans* as the source of the G α_q backbone, combined with C-terminal mammalian G α tails 5, 9 or 21 amino acids in length. cG $\alpha_{q/z5}$ was more promiscuous than any previously described G α construct, supporting receptor activation when co-transfected into *Xenopus* oocytes or mammalian COS-7 cells with most G $\alpha_{1/o-}$, G α_s , and G α_q -coupling receptors. This result was unexpected, and contrasts with the prevailing expectations of experts in the field (Milligan and Rees, 1999). Indeed, the current data (Conklin et al., 1993, 1996; Milligan and Rees, 1999) support the idea that each G protein chimera is only capable of functional interaction with a limited range of receptors. If true, this perceived limitation would necessitate the assay of each GPCR against a panel of chimeric G proteins in order to identify an effective GPCR/G protein combination. The results indicate that certain chimeras, such as cG $\alpha_{q/z5}$, are able to

effectively couple to a very wide number of GPCRs, thus eliminating the need for such multiple assays.

5 *C. elegans* $G\alpha_{q/z5}$ may be used alone or combined with a second chimera such as $cG\alpha_{q/s9}$ to further increase the detection rate especially for $G\alpha_s$ -coupled receptors.

10 Conserved motifs within invertebrate $G\alpha_q$ subunits predict enhanced promiscuity from the use of any invertebrate $G\alpha_q$ backbone, including, but not limited to, the known $G\alpha_q$ sequences listed in Table 8. To provide experimental evidence for this we cloned and expressed a *D. melanogaster* chimera ($dG\alpha_{q/z5}$; Figure 2) containing the five C-terminal amino acids of human $G\alpha_2$. A comparison of $cG\alpha_{q/z5}$, $dG\alpha_{q/z5}$ and $hG\alpha_{q/z5}$ revealed that the two invertebrate chimeras show a similar enhanced coupling to D1 receptors as compared to the corresponding human chimera (Table 9). These data strongly argue against the possibility that *C. elegans* $G\alpha_q$ is somehow unique in its ability to couple promiscuously. Rather, the *D. melanogaster* data suggest that many, if not all, invertebrate $G\alpha_q$ genes may provide a similarly enhanced utility to couple to a wide variety of GPCRs.

25 The general utility of employing $G\alpha$ subunits from primitive organisms may be extended to include non- $G\alpha_q$ subunits from organisms outside of the animal kingdom, including for example, members of the genus *Dictyostelium*. The G-protein α subunits of *Dictyostelium discoideum* do not readily fall into those classes defined for members of the animal kingdom (Wilkie and Yokoyama, 1994), however, individual $G\alpha$ subunits such as G alpha 2 have been

30

shown to directly activate the PLC pathway (Okaichi et al., 1992). Other $G\alpha$ subunits of *Dictyostelium*, such as $G\alpha_4$, may also be useful based on their homology to member of the $G\alpha_q$ family. For example, $G\alpha_4$ exhibits a greater homology to *C. elegans* $G\alpha_q$ than does $G\alpha_2$ (47% vs. 42% at the amino acid level). Therefore, it is anticipated that $G\alpha$ subunits from *Dictyostelium*, with or without amino acid substitutions within the protein, may be useful for functional assays for GPCRs. Therefore, for the purposes of this invention, the term invertebrate $G\alpha_q$ G protein includes *Dictyostelium* $G\alpha_2$ ($G\alpha_2$) and $G\alpha_4$ ($G\alpha_4$) G proteins.

Further enhancements to the coupling scope of the chimeric G proteins described in this invention may be realized by making select point mutations within regions of the protein known to contact GPCRs. For example, amino acids within the α_4 helical domain of $G\alpha_{i1}$ are important for permitting a productive coupling to the 5HT1B receptor (Bae et al., 1999). Mutations altering two amino acids in this domain, Q304 and E308, specifically prevent coupling to 5HT1B. The majority of receptors that did not couple productively to the chimeric G proteins described herein include several members of the 5HT1 subfamily, including 5HT1B. It is predicted, therefore, based on the work of Bae et al. (1999) that making homologous amino acid substitutions in the α_4 region of $cG\alpha_q$ would extend the number of GPCRs that can functionally couple to chimeras, composed of $cG\alpha_q$.

TABLE 8. Description of $G\alpha_q$ subunits from invertebrates useful for construction of chimeras.

SPECIES	COMMON NAME	DESIGNATION	GENBANK ACCESSION NUMBER
<i>Drosophila melanogaster</i>	Fruit fly	GBQ1_drome	P23625
<i>Drosophila melanogaster</i>	Fruit fly	GBQ3_drome	P54400
<i>Limulus polyphemus</i>	Horseshoe crab	GBQ_limpo	g1857923
<i>Patinopecten yessoensis</i>	Scallop	GBQ_patye	O15975
<i>Loligo forbesi</i>	Squid	GBQ_lolfo	P38412
<i>Homarus americanus</i>	Lobster	GBQ_homam	P91950
<i>Lymnaea stagnalis</i>	Pond snail	GBQ_lymst	P38411
<i>Geodia cydonium</i>	Sponge	GBQ_geocy	Y14248
<i>Caenorhabditis elegans</i>	Nematode	GBQ_caeel	AF003739

TABLE 9. Comparison of invertebrate chimerae dG $\alpha_{q/z5}$ and cG $\alpha_{q/z5}$ with two different human hG $\alpha_{q/z5}$ chimerae in their ability to couple to human D1 receptors in COS-7 cells. Ten μ g chimera cDNA and 10 μ g of human D1 receptor cDNA were transfected into COS-7 cells. Transfected cells were monitored for calcium mobilization in the FLIPR™ using the calcium sensitive dye Fluo-3. Maximum agonist concentration was 100 μ M dopamine. Fluorescent data represent the mean from two experiments.

MAXIMAL SIGNAL (FLUORESCENCE UNITS)			
dG $\alpha_{q/z5}$	cG $\alpha_{q/z5}$	hG $\alpha_{q/z5}$ *	hG $\alpha_{q/z5}$ †
2149	4832	0	0

* Identical to Accession number L76256.

† Ala \rightarrow Ser substitution at position 171.

This invention provides a powerful and rapid system for detecting GPCR activation that is obtained when an invertebrate-based G α_q chimera is coupled to a signal amenable to high throughput screening, such as fluorescence-based detection of calcium mobilization. Specific applications would include: 1) high throughput screening and pharmacological analysis of a known GPCR, e.g., drug discovery; 2) screening of ligands against a cloned orphan receptor whose signaling pathways are unknown; and 3) screening of a cDNA library against one or more ligands in an expression cloning paradigm. In each case, this method supports detection of GPCRs from various classes (G $\alpha_{1/o}$, G α_s -, and G α_q -coupling) in a single assay format with greater efficiency and capture rate than previously described methods.

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What is claimed is:

1. An isolated nucleic acid encoding a chimeric G protein, wherein the chimeric G protein comprises
5 an invertebrate G α q G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate
10 G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than five amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein
15 are present at the C-terminus of such vertebrate G protein.
2. A nucleic acid of claim 1, wherein the chimeric G protein comprises an invertebrate G α q G protein
20 from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with
25 the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than two amino acids, provided that at least
30 five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.
3. A nucleic acid of claim 1, wherein the chimeric G
35 protein comprises an invertebrate G α q G protein

from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

4. The nucleic acid of claim 1, wherein the nucleic acid is DNA.
5. The nucleic acid of claim 4, wherein the DNA is cDNA.
6. The nucleic acid of claim 4, wherein the DNA is genomic DNA.
7. The nucleic acid of claim 1, wherein the nucleic acid is RNA.
8. The nucleic acid of claim 1, wherein the vertebrate G protein is a mammalian G protein.
9. The nucleic acid of claim 1, wherein the contiguous amino acids which have been deleted are contained in FVFAAVKDTILQHNLKEYNLV* (SEQ ID NO: 37), wherein V* is the C-terminal amino acid.
10. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate G α z G protein.
11. The nucleic acid of claim 10, wherein the number of contiguous amino acids which have replaced the deleted amino acids are contained in

FVFDAVTDVIIQNNLKYIGLC* (SEQ ID NO: 38), wherein C* is the C-terminal amino acid.

- 5 12. The nucleic acid of claim 10, wherein the invertebrate G α q G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five contiguous amino acids beginning with the C-terminal amino acid of the vertebrate G α z protein.
- 10 13. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate G α s G protein.
- 15 14. The nucleic acid of claim 13, wherein the number of contiguous amino acids which have replaced the deleted amino acids are contained in RVFNDCRDIIQRMHLRQYELL* (SEQ ID NO: 39), wherein
- 20 L* is the C-terminal amino acid.
- 25 15. The nucleic acid of claim 13, wherein the invertebrate G α q G protein has nine contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by nine contiguous amino acids beginning with the C-terminal amino acid of the vertebrate G α s protein.
- 30 16. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate G α i3 G protein.
- 35 17. The nucleic acid of claim 16, wherein the number of contiguous amino acids which have replaced the

deleted amino acids are contained in FVFDAVTDVIIKNNLKECGLY* (SEQ ID NO: 40), wherein Y* is the C-terminal amino acid.

- 5 18. The nucleic acid of claim 16, wherein the invertebrate $G\alpha_q$ G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five
10 contiguous amino acids beginning with the C-terminal amino acid of the vertebrate $G\alpha_{i3}$ protein.
- 15 19. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate $G\alpha_{i1}$ G protein, a vertebrate $G\alpha_{i2}$ G protein, a vertebrate $G\alpha_oA$ G protein, or a vertebrate $G\alpha_oB$ G protein.
- 20 20. The nucleic acid of claim 1, wherein the invertebrate $G\alpha_q$ G protein is a *Caenorhabditis elegans* $G\alpha_q$ G protein.
- 25 21. The nucleic acid of claim 1, wherein the invertebrate $G\alpha_q$ G protein is a *Drosophila melanogaster* $G\alpha_q$ G protein, a *Limulus polyphemus* $G\alpha_q$ G protein, a *Patinopecten yessoensis* $G\alpha_q$ G protein, a *Loligo forbesi* $G\alpha_q$ G protein, a *Homarus americanus* $G\alpha_q$ G protein, a *Lymnaea stagnalis* $G\alpha_q$ G protein, a *Geodia cydonium* $G\alpha_q$ G
30 protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein.

22. The nucleic acid of claim 1, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).
23. A vector comprising the nucleic acid of claim 1.
24. A vector of claim 23 adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the chimeric G protein so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect, or mammalian cell.
25. The vector of claim 24, wherein the vector is a plasmid, a baculovirus, or a retrovirus.
26. A cell comprising the vector of claim 23, wherein the cell comprises DNA encoding a mammalian G protein-coupled receptor.
27. A cell of claim 26, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

28. A cell of claim 26, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.
- 5 29. A cell of claim 26, wherein the cell is a non-mammalian cell.
30. A cell of claim 29, wherein the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.
- 10 31. A cell of claim 26, wherein the cell is a mammalian cell.
- 15 32. A mammalian cell of claim 31, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.
- 20 33. A cell of claim 26, wherein the cell is an insect cell.
34. An insect cell of claim 33, wherein the insect cell is an Sf9 cell, an Sf21 cell or a *Trichoplusia ni* 5B-4 cell.
- 25 35. A membrane preparation isolated from the cell of any one of claims 26, 27, 28, 29, 31, 32, 33 or 34.
- 30 36. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the
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compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

37. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

38. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

39. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.
40. The process of claim 36, 37, 38, or 39, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.
41. The process of claim 36, 37, 38, or 39, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.
42. The process of claim 36, 37, 38, or 39, wherein the mammalian G protein-coupled receptor is a human Y5 receptor, a human GALR2 receptor, a human kappa opioid receptor, a human NPFF1 receptor, a human NPFF2 receptor, a human α 2A adrenergic receptor, a human dopamine D2 receptor, a human GALR1 receptor, a human Y2 receptor, a human Y1 receptor, a human Y4 receptor, a human α 1A adrenergic receptor, a human dopamine D1 receptor, or a rat NTR1 receptor.

43. A process for determining whether a chemical compound specifically binds to and activates a mammalian G protein-coupled receptor, which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor.
44. The process of claim 43, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.
45. The process of claim 43, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.
46. The process of claim 43, wherein the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element.
47. The process of claim 43, wherein the second messenger response is measured by a change in cell proliferation.

48. The process of claim 43, wherein the second messenger response is a $G\alpha_q$ second messenger response.

5 49. The process of claim 48, wherein the $G\alpha_q$ second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate.

10 50. The process of claim 48, wherein the $G\alpha_q$ second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid.

15 51. The process of claim 48, wherein the $G\alpha_q$ second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation.

20 52. The process of claim 48, wherein the $G\alpha_q$ second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium.

25 53. The process of claim 52, wherein the measure of intracellular calcium levels is made by chloride current readings.

30 54. The process of claim 52, wherein the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the

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detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

- 5 55. A process for determining whether a chemical
 compound specifically binds to and inhibits
 activation of a mammalian G protein-coupled
 receptor, which comprises separately contacting
10 cells producing a second messenger response,
 expressing the DNA encoding the mammalian G
 protein-coupled receptor, and expressing the DNA
 encoding a chimeric G protein, wherein such
 cells do not normally express the DNA encoding
15 the chimeric G protein, with both the chemical
 compound and a second chemical compound known to
 activate the mammalian G protein-coupled
 receptor, and with only the second chemical
 compound, under conditions suitable for
20 activation of the mammalian G protein-coupled
 receptor, and measuring the second messenger
 response in the presence of only the second
 chemical compound and in the presence of both
 the second chemical compound and the chemical
25 compound, a smaller change in the second
 messenger response in the presence of both the
 chemical compound and the second chemical
 compound than in the presence of only the second
 chemical compound indicating that the chemical
30 compound inhibits activation of the mammalian G
 protein-coupled receptor.

56. The process of claim 55, wherein the DNA encoding
 the mammalian G protein-coupled receptor is
 endogenous to the cell.

57. The process of claim 55, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

5 58. The process of claim 55, wherein the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element.

10 59. The process of claim 55, wherein the second messenger response is measured by a change in cell proliferation.

15 60. The process of claim 55, wherein the second messenger response is a $G\alpha_q$ second messenger response.

20 61. The process of claim 60, wherein the $G\alpha_q$ second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

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30 62. The process of claim 60, wherein the $G\alpha_q$ second messenger response comprises activation of MAP kinase and the change in second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

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- 5 63. The process of claim 60, wherein the Gαq second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.
- 10 64. The process of claim 60, wherein the Gαq second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.
- 15 65. The process of claim 64, wherein the measure of intracellular calcium levels is made by chloride current readings.
- 20 66. The process of claim 64, wherein the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.
- 25 67. A process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a compound which activates the mammalian G protein-coupled receptor which comprises:
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(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor.

68. A process of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian G protein-coupled receptor to identify a compound which inhibits the activation of the mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds in the presence of a known mammalian G protein-coupled

receptor agonist, under conditions
permitting activation of the mammalian G
protein-coupled receptor;

5 (b) determining whether the extent or amount of
activation of the mammalian G protein-
coupled receptor is reduced in the presence
of one or more of the compounds, relative
10 to the extent or amount of activation of
the mammalian G protein-coupled receptor in
the absence of such one or more compounds;
and if so

15 (c) separately determining whether each such
compound inhibits activation of the
mammalian G protein-coupled receptor for
each compound included in the plurality of
compounds, so as to thereby identify any
20 compound included in such plurality of
compounds which inhibits the activation of
the mammalian G protein-coupled receptor.

69. The process of claim 67 or 68, wherein the DNA
encoding the mammalian G protein-coupled receptor
25 is endogenous to the cell.

70. The process of claim 67 or 68, wherein the DNA
encoding the mammalian G protein-coupled receptor
is transfected into the cell.

30 71. A process for determining whether a chemical
compound is a mammalian G protein-coupled
receptor agonist, which comprises separately
contacting membrane preparations from cells
35 transfected with and expressing DNA encoding a
chimeric G protein and expressing DNA encoding a

mammalian G protein-coupled receptor with both the compound and [³⁵S]GTPγS, and with only [³⁵S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [³⁵S]GTPγS binding to the membrane preparation and an increase in [³⁵S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

72. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound, [³⁵S]GTPγS, and a second chemical compound known to activate the mammalian G protein-coupled receptor, with [³⁵S]GTPγS and only the second compound, and with [³⁵S]GTPγS alone, under conditions permitting the activation of the mammalian G protein-coupled receptor, detecting [³⁵S]GTPγS binding to each membrane preparation, comparing the increase in [³⁵S]GTPγS binding in the presence of the compound and the second compound relative to the binding of [³⁵S]GTPγS alone to the increase in [³⁵S]GTPγS binding in the presence of the second chemical compound relative to the binding of [³⁵S]GTPγS alone, and detecting a smaller increase in [³⁵S]GTPγS binding in the presence of the compound and the second compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

73. The process of claim 71 or 72, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.
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74. The process of claim 71 or 72, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.
- 10
75. The process of claim 71 or 72, wherein the mammalian G protein-coupled receptor produces a $G\alpha s$ second messenger response in the absence of the chimeric G protein.
- 15
76. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.
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77. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled
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receptor with the chemical compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

78. The process of claim 76 or 77, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

79. The process of claim 76 or 77, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

80. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

81. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning

with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha z$ protein, wherein such number equals the number of amino acids deleted.

82. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate $G\alpha q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha s$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha s$ protein, wherein such number equals the number of amino acids deleted.

83. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate $G\alpha q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha i3$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha i3$ protein, wherein such number equals the number of amino acids deleted.

84. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises a *Caenorhabditis elegans* $G\alpha q$ G protein from which at least five,

but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

85. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises a *Drosophila melanogaster* G α_q G protein, a *Limulus polyphemus* G α_q G protein, a *Patinopecten yessoensis* G α_q G protein, a *Loligo forbesi* G α_q G protein, a *Homarus americanus* G α_q G protein, a *Lymnaea stagnalis* G α_q G protein, a *Geodia cydonium* G α_q G protein, or a *Dictyostelium discoideum* G α_q G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of a vertebrate G protein, wherein such number equals the number of amino acids deleted.

86. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* G $\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* G $\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* G $\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* G $\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* G $\alpha_{q/i3(5)}$ (SEQ ID

NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$
(SEQ ID NO: 41).

5 87. The process of any one of claims 36, 37, 38, 39,
 43, 55, 67, 68, 71, 72, 76, or 77, wherein the
 cell is an insect cell.

 88. The process of any one of claims 36, 37, 38, 39,
 43, 55, 67, 68, 71, 72, 76, or 77, wherein the
10 cell is a mammalian cell.

 89. The process of claim 88, wherein the cell is
 nonneuronal in origin.

15 90. The process of claim 89, wherein the nonneuronal
 cell is a COS-7 cell, 293 human embryonic kidney
 cell, a CHO cell, a NIH-3T3 cell, a mouse Y1
 cell, or a LM(tk-) cell.

20 91. A process for identifying a chemical compound
 which specifically binds to a mammalian G
 protein-coupled receptor which comprises
 contacting cells transfected with and expressing
 DNA encoding a chimeric G protein and expressing
25 DNA encoding a mammalian G protein-coupled
 receptor, wherein such cells do not normally
 express the DNA encoding the chimeric G protein,
 with the compound under conditions suitable for
 binding, and detecting specific binding of the
30 chemical compound to the mammalian G protein-
 coupled receptor.

 92. A process for identifying a chemical compound
 which specifically binds to a mammalian G
35 protein-coupled receptor which comprises
 contacting a membrane preparation from cells

transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

93. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

94. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-

coupled receptor which comprises separately contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

95. A process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound known to bind specifically to the mammalian G protein-coupled receptor;

(b) contacting the cells of step (a) with the plurality of compounds not known to

bind specifically to the mammalian G protein-coupled receptor, under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(c) determining whether the binding of the compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

96. A process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor under conditions

permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

5 (b) determining whether the binding of a compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of
10 the compound in the absence of the plurality of compounds; and if so

 (c) separately determining the binding to the mammalian G protein-coupled
15 receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

20 97. The process of claim 91, 92, 93, 94, 95, or 96, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

25 98. The process of claim 91, 92, 93, 94, 95, or 96, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

30 99. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an invertebrate G α q G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and
35 replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with

the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

5 100. The process of any one of claims 91, 92, 93, 94,
95, or 96, wherein the chimeric G protein
comprises an invertebrate G α q G protein from
which at least five, but not more than twenty-
one, contiguous amino acids beginning with the
10 C-terminal amino acid have been deleted and
replaced by a number of contiguous amino acids
present in a vertebrate G α z protein beginning
with the C-terminal amino acid of such
vertebrate G α z protein, wherein such number
15 equals the number of amino acids deleted.

20 101. The process of any one of claims 91, 92, 93, 94,
95, or 96, wherein the chimeric G protein
comprises an invertebrate G α q G protein from
which at least five, but not more than twenty-
one, contiguous amino acids beginning with the
C-terminal amino acid have been deleted and
replaced by a number of contiguous amino acids
present in a vertebrate G α s protein beginning
25 with the C-terminal amino acid of such
vertebrate G α s protein, wherein such number
equals the number of amino acids deleted.

30 102. The process of any one of claims 91, 92, 93, 94,
95, or 96, wherein the chimeric G protein
comprises an invertebrate G α q G protein from
which at least five, but not more than twenty-
one, contiguous amino acids beginning with the
C-terminal amino acid have been deleted and
35 replaced by a number of contiguous amino acids

present in a vertebrate $G\alpha i3$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha i3$ protein, wherein such number equals the number of amino acids deleted.

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103. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an *Caenorhabditis elegans* $G\alpha q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

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104. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises a *Drosophila melanogaster* $G\alpha q$ G protein, a *Limulus polyphemus* $G\alpha q$ G protein, a *Patinopecten yessoensis* $G\alpha q$ G protein, a *Loligo forbesi* $G\alpha q$ G protein, a *Homarus americanus* $G\alpha q$ G protein, a *Lymnaea stagnalis* $G\alpha q$ G protein, a *Geodia cydonium* $G\alpha q$ G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

105. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).
106. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the cell is an insect cell.
107. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the cell is a mammalian cell.
108. The process of claim 107, wherein the cell is nonneuronal in origin.
109. The process of claim 108, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.
110. The process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using the process of any of claims 36, 37, 43, 67, 71, or 76 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

111. The process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using the process of any of claims 38, 39, 55, 68, 72, or 77 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.
112. The process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using the process of any of claims 91, 92, 93, 94, 95, or 96 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.
113. The process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 36, 37, 43, 67, 71, or 76 or a novel structural and functional analog or homolog thereof.
114. The process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 38, 39, 55, 68, 72, or 77 or a novel structural and functional analog or homolog thereof.
115. The process for preparing a composition which comprises admixing a carrier and a

pharmaceutically effective amount of a chemical compound identified by the process of any of claims 91, 92, 93, 94, 95, or 96 or a novel structural and functional analog or homolog thereof.

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116. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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117. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

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118. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting
5 cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding
10 the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second
15 messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.
119. The process of claim 118, wherein the second messenger response is a $G\alpha_q$ second messenger response.
120. The process of claim 119, wherein the $G\alpha_q$ second messenger response comprises intracellular
25 calcium levels and the change in second messenger is an increase in the measure of intracellular calcium.
121. The process of claim 120, wherein the measure of intracellular calcium levels is made by chloride
30 current readings.

122. The process of claim 120, wherein the measure of
intracellular calcium is made by fluorescence
readings, luminescence readings,
electrophysiological readings, or through the
5 detection of a reporter protein under the
transcriptional control of a calcium-responsive
promoter element.

123. A process of screening a plurality of chemical
10 compounds not known to activate a mammalian G
protein-coupled receptor to identify a ligand
for the mammalian G protein-coupled receptor
which comprises:

15 (a) contacting cells transfected with and
expressing DNA encoding a chimeric G
protein and expressing DNA encoding a
mammalian G protein-coupled receptor with
the plurality of compounds not known to
20 activate the mammalian G protein-coupled
receptor, under conditions permitting
activation of the mammalian G protein-
coupled receptor;

25 (b) determining whether the activity of the
mammalian G protein-coupled receptor is
increased in the presence of one or more of
the compounds; and if so

30 (c) separately determining whether the
activation of the mammalian G protein-
coupled receptor is increased by any
compound included in the plurality of
compounds, so as to thereby identify each
35 compound which activates the mammalian G
protein-coupled receptor and is a ligand

for the mammalian G protein-coupled receptor.

124. A process for determining whether a chemical
5 compound is a ligand for a mammalian G protein-coupled receptor, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a
10 mammalian G protein-coupled receptor with both the compound and [³⁵S]GTPγS, and with only [³⁵S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [³⁵S]GTPγS binding to the
15 membrane preparation and an increase in [³⁵S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G
20 protein-coupled receptor.

125. A process for determining whether a chemical
25 compound is a ligand for the mammalian G protein-coupled receptor, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G
30 protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the
35 chemical compound activates the mammalian G

protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

5 126. A process for identifying a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not
10 normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating
15 that the compound is a ligand for the mammalian G protein-coupled receptor.

20 127. A process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein
25 such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled
30 receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

35 128. The process of claim 116, 117, 118, 123, 124, 125, 126, or 127, wherein the DNA encoding the

mammalian G protein-coupled receptor is endogenous to the cell.

5 129. The process of claim 116, 117, 118, 123, 124, 125, 126, or 127, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

10 130. The process of any one claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

15 20 25 30 131. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_z$ protein, wherein such number equals the number of amino acids deleted.

35 132. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric

G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha_s$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha_s$ protein, wherein such number equals the number of amino acids deleted.

133. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an invertebrate $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $Gi3$ protein beginning with the C-terminal amino acid of such vertebrate $Gi3$ protein, wherein such number equals the number of amino acids deleted.

134. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an *Caenorhabditis elegans* $G\alpha_q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

135. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises a *Drosophila melanogaster* $G\alpha_q$ G protein, a *Limulus polyphemus* $G\alpha_q$ G protein, a *Patinopecten yessoensis* $G\alpha_q$ G protein, a *Loligo forbesi* $G\alpha_q$ G protein, a *Homarus americanus* $G\alpha_q$ G protein, a *Lymnaea stagnalis* $G\alpha_q$ G protein, a *Geodia cydonium* $G\alpha_q$ G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.
136. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).
137. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the cell is an insect cell.

138. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the cell is a mammalian cell.

5 139. The process of claim 138, wherein the cell is nonneuronal in origin.

10 140. The process of claim 139, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

15 141. A process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

20 (a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting
25 activation of a mammalian G protein-coupled receptor;

30 (b) determining whether the ligand activates the cells expressing the plurality of independent clones and the chimeric G protein; and if so

35 (c) isolating the single clone which expresses the mammalian G protein-coupled receptor activated by the ligand, so as to thereby identify any clone included in the

plurality of clones as encoding a mammalian G protein-coupled receptor.

142. A process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting specific binding to a mammalian G protein-coupled receptor;

(b) determining whether the ligand specifically binds to the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor which specifically binds to the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

143. The process of claim 141 or 142, wherein the DNA encoding the plurality of independent clones is endogenous to the cell.

144. The process of claim 141 or 142, wherein the DNA encoding the plurality of independent clones is transfected into the cell.

5 145. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate $G\alpha q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have
10 been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

15 146. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate $G\alpha q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids
20 beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate $G\alpha z$ protein beginning with the C-terminal amino acid of such vertebrate $G\alpha z$ protein, wherein
25 such number equals the number of amino acids deleted.

30 147. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate $G\alpha q$ G protein from which at least five, but not more than twenty-one, contiguous amino acids
beginning with the C-terminal amino acid have been deleted and replaced by a number of
contiguous amino acids present in a vertebrate
35 $G\alpha s$ protein beginning with the C-terminal amino

acid of such vertebrate $G\alpha s$ protein, wherein such number equals the number of amino acids deleted.

5 148. The process of claim 141 or 142, wherein the
chimeric G protein comprises an invertebrate $G\alpha q$
G protein from which at least five, but not more
than twenty-one, contiguous amino acids
beginning with the C-terminal amino acid have
10 been deleted and replaced by a number of
contiguous amino acids present in a vertebrate
 $G\alpha i3$ protein beginning with the C-terminal amino
acid of such vertebrate $G\alpha i3$ protein, wherein
such number equals the number of amino acids
15 deleted.

149. The process of claim 141 or 142, wherein the
chimeric G protein comprises an *Caenorhabditis*
elegans $G\alpha q$ G protein from which at least five,
but not more than twenty-one, contiguous amino
20 acids beginning with the C-terminal amino acid
have been deleted and replaced by a number of
contiguous amino acids present in a vertebrate G
protein beginning with the C-terminal amino acid
of such vertebrate G protein, wherein such
25 number equals the number of amino acids deleted.

150. The process of 141 or 142, wherein the chimeric
G protein comprises a *Drosophila melanogaster*
 $G\alpha q$ G protein, a *Limulus polyphemus* $G\alpha q$ G
30 protein, a *Patinopecten yessoensis* $G\alpha q$ G
protein, a *Loligo forbesi* $G\alpha q$ G protein, a
Homarus americanus $G\alpha q$ G protein, a *Lymnaea*
stagnalis $G\alpha q$ G protein, a *Geodia cydonium* $G\alpha q$ G
35 protein, or a *Dictyostelium discoideum* $G\alpha_4$ G

protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

10 151. The process of claim 141 or 142, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/13(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

20 152. The process of claim 141 or 142, wherein the cell is an insect cell.

25 153. The process of claim 141 or 142, wherein the cell is a mammalian cell.

154. The process of claim 153, wherein the cell is nonneuronal in origin.

30 155. The process of claim 154, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

CHIMERIC G PROTEINS AND USES THEREOF**Abstract Of The Disclosure**

5

10 This invention provides isolated nucleic acids encoding chimeric G proteins, vectors comprising nucleic acids encoding chimeric G proteins, cells comprising such vectors, processes of determining agonists and antagonists of mammalian G protein-coupled receptors utilizing chimeric G proteins, processes of determining compounds which bind to mammalian G protein-coupled receptors utilizing chimeric G proteins, processes for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor utilizing chimeric G proteins, processes for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by a process of the invention utilizing chimeric G proteins, processes of identifying a ligand for a mammalian G protein-coupled receptor utilizing chimeric G proteins, and processes of screening a plurality of independent clones to identify and isolate a clone encoding a mammalian G protein-coupled receptor utilizing chimeric G proteins.

25

FIGURE 1



FIGURE 2A

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C. elegans $G\alpha_{q/z5}$

```

1  MACCLSEEAR EQKRINQEIE KQLQORDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAADVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYSDDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVASE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQHNLK YIGLC

```

C. elegans $G\alpha_{q/z9}$

```

1  MACCLSEEAR EQKRINQEIE KQLQORDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAADVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYSDDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVASE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQNNLK YIGLC

```

C. elegans $G\alpha_{q/s9}$

```

1  MACCLSEEAR EQKRINQEIE KQLQORDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAADVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYSDDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVASE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQMHLR QYELL

```

C. elegans $G\alpha_{q/s21}$

```

1  MACCLSEEAR EQKRINQEIE KQLQORDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAADVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYSDDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVASE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRRVFNDC RDIIQRMHLR QYELL

```

C. elegans $G\alpha_{q/13(5)}$

```

1  MACCLSEEAR EQKRINQEIE KQLQORDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAADVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYSDDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVASE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQHNLK ECGLY

```

D. melanogaster $G\alpha_{q/z5}$

```
1 MECCLSEEAK EQKRINQEIE KQLRRDKRDA RRELKLLLLG TGESGKSTFI KQMRIIHGSG
61 YSDEDKRGYI KLVFQNI FMA MQSMIKAMDM LKISYGQGEH SELADLVMSI DYETVTTFED
121 PYLNAIKTLW DDAGIQECYD RRREYQLTDS AKYYLKDLDR VAQPAYLPTE QDILRVRVPT
181 TGIIEYPFDL EEIRFRMVDV GGQSRERRKW IHCFENVTSI IFLVASEYD QILFESDNEN
241 RMEESKALFR TIITYPWFQN SSVILFLNKK DLLEEKIMYS HLVDYFPEYD GPQRDAITAR
301 EFILRMFVDL NPDSEKIIYS HFTCATDTEN IRFVFAAVKD TILQSNLKYI GLC
```

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FIGURE 3

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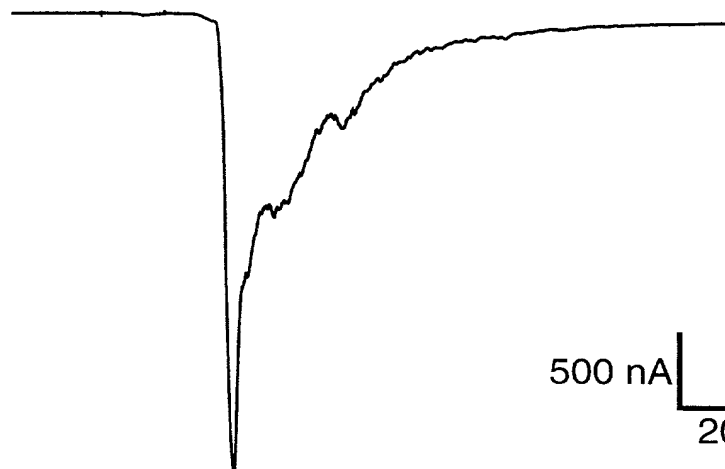
hNPFF1

NPFF 1 μ M



hNPFF1 + cG $\alpha_{q/z5}$

NPFF 1 μ M



500 nA

20 s

hNPFF1 + cG $\alpha_{q/z5}$
+ 190 ng EGTA

NPFF 1 μ M



FIGURE 4

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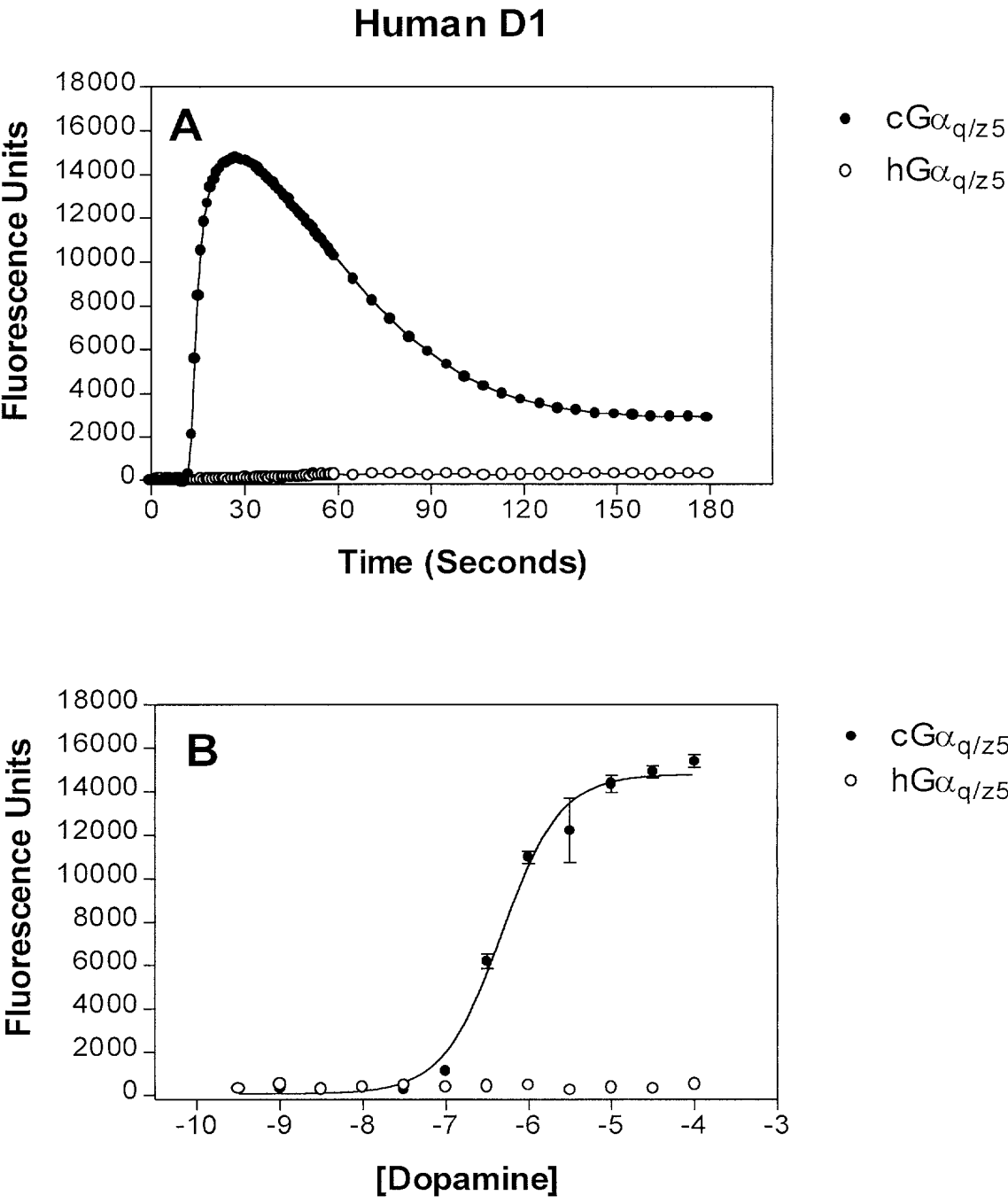


FIGURE 5A

```

      *           20           *           40           *
GBQ_HUMAN : MTLESIMACCLSEEAKEARRINDEIERQRRDKRDARRELKLLLLGTGES
GBQ_CANFA : MTLESIMACCLSEEAKEARRINDEIERQRRDKRDARRELKLLLLGTGES
GBQ_MOUSE : MTLESIMACCLSEEAKEARRINDEIERHVRRRDKRDARRELKLLLLGTGES
GBQ_XENLA : MTLESIMACCLSEEAKEARRINDEIERQRRDKRDARRELKLLLLGTGES
GBQ_PATYE : ~~~~~MACCLSEEAKQKRINCEIEKELRKAKNDARRELKLLLLGTGES
GBQ_LYMST : ~~~~~MACCLIPDELKQKRINQEIERQKRDKRDARRELKLLLLGTGES
GBQ1_DROM : ~~~~~MECCLSEEAKQKRINQEIEKQRRDKRDARRELKLLLLGTGES
GBQ3_DROM : ~~~~~MECCLSEEAKQKRINQEIEKQRRDKRDARRELKLLLLGTGES
GBQ_HOMAM : ~~~~~MACCLSEEAKQKRINQEIERQRKDKRDARRELKLLLLGTGES
GBQ_LIMPO : ~~~~~MACCLSEEGKQKRINQEIERQRKDKRDARRELKLLLLGTGES
GBQ_LOLFO : ~~~~~MACCLSEEAKQKRINQEIEKQRRDKRDARRELKLLLLGTGES
GBQ_CAEL : ~~~~~MACCLSEEARQKRINQEIEKQQRDKNDARRELKLLLLGTGES
GBQ_GEOCY : ~~~~~MSCLSEEARLQKRINTRINREQRHKDKKEIKLLLLGTGES

      60           *           80           *           100
GBQ_HUMAN : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_CANFA : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_MOUSE : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_XENLA : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_PATYE : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_LYMST : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ1_DROM : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ3_DROM : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_HOMAM : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_LIMPO : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_LOLFO : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_CAEL : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP
GBQ_GEOCY : GKSTFIKQMRIIHGSSGYSDEDKRGFTKLYNIETMARAVDTEKIP

      *           120           *           140           *
GBQ_HUMAN : KYEHNK--AHQLVREVVVKS---AENPYVDKSWNDPGIQEC
GBQ_CANFA : KYEHNK--AHQLVREVVVKS---AENPYVDKSWNDPGIQEC
GBQ_MOUSE : KYEHNK--AHQLVREVVVKS---AENPYVDKSWNDPGIQEC
GBQ_XENLA : KYEHNK--GHQLVREVVVKA---SENPYVDKSWNDPGIQEC
GBQ_PATYE : FEVADNE--ENIMIRQVYETT---TLDSQSVEILSWADAGIQEC
GBQ_LYMST : INPANR--ENGNMIRQYETT---TLDKPCVDISWNDDGIQEC
GBQ1_DROM : GQGEHS--ELDLVMSIYETT---TLDEPYLNKTWDDAGIQEC
GBQ3_DROM : GQGEHS--ELDLVMSIYETT---TLDEPYLNKTWDDAGIQEC
GBQ_HOMAM : GDSANI--EHLVRSVYETS---TLDEPYVTMNSWQDTGIQEC
GBQ_LIMPO : KDRNNI--ENELVLSVYETT---TLDSPYVEKSWVDPGIQEC
GBQ_LOLFO : KDPSN--NEHEFLNSIADSAD---IEDGHVTKGCVTDPGMQEC
GBQ_CAEL : FGNSEELQEKAVVREVFST---SEEPYVSYKEWEDSGEQEC
GBQ_GEOCY : IDPD--AQRHVQLLMALRPSTAQ---SLGGETCERKWQDAGIQEC

```

FIGURE 5B

GBQ_HUMAN : YDRRREYC-LSDSTKYYINLDRVADPAYLFTQQDVLRVRVPTTIGIIEYF
 GBQ_CANFA : YDRRREYC-LSDSTKYYINLDRVADPAYLFTQQDVLRVRVPTTIGIIEYF
 GBQ_MOUSE : YDRRREYC-LSDSTKYYINLDRVADPSYLFTQQDVLRVRVPTTIGIIEYF
 GBQ_XENLA : YDRRREYC-LSDSTKYYINLDRVADPSYLFTQQDVLRVRVPTTIGIIEYF
 GBQ_PATYE : YDRRREYC-LTDSAKYYLDAVDRIAEPNYLFTLQDILRVRVPTTIGIIEYF
 GBQ_LYMST : YDRRREYC-LTDSAKYYLDSVERISQQDYLFTLQDILRVRVPTTIGIIEYF
 GBQ1_DROM : YDRRREYC-LTDSAKYYLSTLARIEQADYLFTQDILRVRVPTTIGIIEYF
 GBQ3_DROM : YDRRREYC-LTDSAKYYLKLDLRAQPAYLFTQDILRVRVPTTIGIIEYF
 GBQ_HOMAM : YDRRREYC-LTDSAKYYLTLDRIAADKYVSTLQDILRVRVPTTIGIIEYF
 GBQ_LIMPO : YDRRREYC-LTDSAKYYLNDRIAAPNYLFTQDILRVRVPTTIGIIEYF
 GBQ_LOLFO : YDRRREYC-LTDSAKYYLDSVERIHEPGYFTLQDILRVRVPTTIGIIEYF
 GBQ_CAEEL : YDRRREYC-LTDSAKYYLSDLRRLAVPDYFTQDILRVRVPTTIGIIEYF
 GBQ_GEOCY : YDRRREYC-LSDSTKYYINLDRVADPAYLFTQQDVLRVRVPTTIGIIEYF

GBQ_HUMAN : FDLQSVIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_CANFA : FDLQSVIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_MOUSE : FDLQSVIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_XENLA : FDLQSVIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_PATYE : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_LYMST : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ1_DROM : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ3_DROM : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_HOMAM : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_LIMPO : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_LOLFO : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_CAEEL : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD
 GBQ_GEOCY : FDLDSIIFRMVDVGGQSRERKWIICFENVTSIMFVAISEYDQVLVESD

GBQ_HUMAN : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_CANFA : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_MOUSE : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_XENLA : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_PATYE : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_LYMST : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ1_DROM : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ3_DROM : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_HOMAM : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_LIMPO : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_LOLFO : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_CAEEL : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF
 GBQ_GEOCY : N-ENRMEEKALFRITITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDF

FIGURE 5C

* 320 * 340 *

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GBQ_HUMAN : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_CANFA : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_MOUSE : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_XENLA : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_PATYE : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_LYMST : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ1_DROM : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ3_DROM : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_HOMAM : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_LIMPO : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_LOLFO : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_CAEEL : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA
GBQ_GEOCY : PEYDGFQRDAQAAREETIKMFVDNPDSDNIITYSHETCATDTENIRVFVFA

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360 *

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GBQ_HUMAN : AVKDTILQLNLKEYNLV ~~~ : 359
GBQ_CANFA : AVKDTILQLNLKEYNLV ~~~ : 359
GBQ_MOUSE : AVKDTILQLNLKEYNLV ~~~ : 359
GBQ_XENLA : AVKDTILQLNLKEYNLV ~~~ : 359
GBQ_PATYE : AVKDTILQLNLKEYNLV ~~~ : 353
GBQ_LYMST : AVKDTILQLNLKEYNLV ~~~ : 353
GBQ1_DROM : AVKDTILQLNLKEYNLV ~~~ : 353
GBQ3_DROM : AVKDTILQLNLKEYNLV ~~~ : 353
GBQ_HOMAM : AVKDTILQLNLKEYNLV ~~~ : 353
GBQ_LIMPO : AVKDTILQLNLKEYNLV ~~~ : 353
GBQ_LOLFO : AVKDTILQLNLKEYNLV ~~~ : 354
GBQ_CAEEL : AVKDTILQLNLKEYNLV ~~~ : 355
GBQ_GEOCY : AVKDTILQLNLKEYNLV ~~~ : 355

```


As a below-named inventor, I hereby declare that:

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

the specification of which:
(check one)

_____ was filed on _____ as _____

and was amended December 23, 1999
(if applicable)

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56

Prior Foreign Application(s)

Priority Claimed

[illegible]

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

<u>Provisional Application No.</u>	<u>Filing Date</u>	<u>Status</u>
N/A		

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

<u>Application Serial No.</u>	<u>Filing Date</u>	<u>Status</u>
N/A		

And I hereby appoint

John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); Jay H. Maioli (Reg. No. 27,213); William E. Pelton (Reg. No. 25, 702); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29, 691) Wendy E. Miller (Reg. No. 35, 615); Richard S. Milner (Reg. No. 33,970); Robert T. Maldonado (Reg. No. 38,232); Paul Teng (Reg. No. 40, 837); Gary J. Gershik (Reg. No. 39,992); Richard F. Jaworski (Reg. No. 33,515); Elizabeth M. Wieckowski (Reg. No. 42,226); and Pedro C. Fernandez (Reg. No. 41,741)

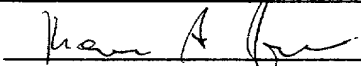
and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone call, regarding this application to

John P. White, Esq. Reg. No. 28,678
Cooper & Dunham LLP
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New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor Kenneth A. Jones

Inventor's signature 

Citizenship U.S.A. Date of signature December 22, 1999

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Post Office Address same as residence address

Full name of sole or first joint inventor Mary W. Walker

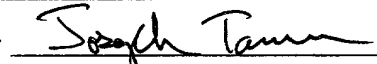
Inventor's signature 

Citizenship U.S.A. Date of signature December 20, 1999

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Post Office Address same as residence address

Full name of sole or first joint inventor Joseph Tamm

Inventor's signature 

Citizenship U.S.A. Date of signature 12/22/99

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Post Office Address same as residence address

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inventor (if any) Theresa A. Branchek

Inventor's signature Theresa A. Branchek

Citizenship U.S.A. Date of signature 12/22/99

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Post Office Address same as residence address

Full name of joint
inventor (if any) Christophe P.G. Gerald

Inventor's signature Christophe P.G. Gerald

Citizenship France Date of signature 12/22/99

Residence 204B Union Street, Ridgewood, New Jersey 07450, U.S.A.

Post Office Address same as residence address

Full name of joint
inventor (if any) _____

Inventor's signature _____

Citizenship _____ Date of signature _____

Residence _____

Post Office Address _____

SEQUENCE LISTING

<110> Jones, Kenneth A.

Walker, Mary W.

Tamm, Joseph

Branchek, Theresa A.

Gerald, Christophe P.G.

<120> Chimeric G-Proteins And Uses Thereof

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 50 55 60

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 65 70 75 80

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 Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe
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 Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr
 245 250 255
 Tyr Pro Trp Phe Thr Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys
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 Asp Leu Leu Glu Glu Lys Ile Leu Tyr Ser His Leu Ala Asp Tyr Phe
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 Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Arg Val
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 Gly Leu Tyr
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 Ser Gly Tyr Ser Asp Glu Asp Lys Arg Gly Phe Thr Lys Leu Val Tyr
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 Leu Lys Ile Pro Tyr Lys Tyr Glu His Asn Lys Ala His Ala Gln Leu
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 Val Arg Glu Val Asp Val Glu Lys Val Ser Ala Phe Glu Asn Pro Tyr
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 Val Asp Ala Ile Lys Ser Leu Trp Asn Asp Pro Gly Ile Gln Glu Cys
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Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr
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 Asp Lys Ile Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn
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50 55 60

Ser Gly Tyr Ser Asp Glu Asp Lys Arg Gly Phe Thr Lys Leu Val Tyr
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Gln Asn Ile Phe Thr Ala Met Gln Ala Met Ile Arg Ala Met Asp Thr
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Leu Lys Ile Pro Tyr Lys Tyr Glu His Asn Lys Ala His Ala Gln Leu
100 105 110

Val Arg Glu Val Asp Val Glu Lys Val Ser Ala Phe Glu Asn Pro Tyr
115 120 125

Val Asp Ala Ile Lys Ser Leu Trp Asn Asp Pro Gly Ile Gln Glu Cys
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Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr
145 150 155 160

Leu Asn Asp Leu Asp Arg Val Ala Asp Pro Ala Tyr Leu Pro Thr Gln
165 170 175

Gln Asp Val Leu Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr
180 185 190

Pro Phe Asp Leu Gln Ser Val Ile Phe Arg Met Val Asp Val Gly Gly
195 200 205

Gln Arg Ser Glu Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr
210 215 220

Ser Ile Met Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val
225 230 235 240

Glu Ser Asp Asn Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg
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Thr Ile Ile Thr Tyr Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe
260 265 270

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Ser Ile Met Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val
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Glu Ser Asp Asn Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg
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Thr Ile Ile Thr Tyr Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe
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Leu Asn Lys Lys Asp Leu Leu Glu Glu Lys Ile Met Tyr Ser His Leu
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 305 310 315 320

Asp Lys Ile Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn
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Arg	Met	Glu	Glu	Ser	Lys	Ala	Leu	Phe	Arg	Thr	Ile	Ile	Thr	Tyr	Pro	245	250	255	
Trp	Phe	Gln	Asn	Ser	Ser	Val	Ile	Leu	Phe	Leu	Asn	Lys	Lys	Asp	Leu	260	265	270	
Leu	Glu	Glu	Lys	Ile	Met	His	Ser	His	Leu	Val	Asp	Tyr	Phe	Pro	Glu	275	280	285	
Phe	Asp	Gly	Gln	Lys	Lys	Asp	Ala	Gln	Gly	Ala	Arg	Glu	Phe	Ile	Leu	290	295	300	
Arg	Met	Phe	Val	Asp	Leu	Asn	Pro	Asp	Pro	Asp	Lys	Ile	Ile	Tyr	Ser	305	310	315	320

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Val

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<212> PRT
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Asp Lys Arg Ser His Ile Lys Ile Val Tyr Gln Asn Ile Phe Met Ala
65 70 75 80

Met His Ala Met Ile Arg Ala Met Asp Thr Leu Asn Ile Gln Tyr Ile
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Asn Pro Ala Asn Arg Glu Asn Gly Asn Met Ile Arg Gln Ile Asp Tyr
100 105 110

Glu Thr Val Thr Thr Phe Asp Lys Pro Cys Val Asp Ala Ile Ile Ser
115 120 125

Leu Trp Asn Asp Asp Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
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Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Asp Ser Val Glu Arg
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Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg					
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Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe Leu Val					
	210		215		220
Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Ser Asp Asn Glu Asn					
	225		230		240
Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro					
	245		250		255
Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu					
	260		265		270
Leu Glu Glu Lys Ile Met His Ser His Leu Val Asp Tyr Phe Pro Glu					
	275		280		285
Phe Asp Gly Pro Lys Lys Glu Ala Ser Thr Ala Arg Glu Phe Ile Leu					
	290		295		300
Lys Met Phe Val Glu Leu Asn Pro Asp Pro Asp Lys Ile Ile Tyr Ser					
	305		310		320
His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala					
	325		330		335
Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn Leu					
	340		345		350

Val

<210> 12

<211> 353

<212> PRT

<213> Drosophila melanogaster

<400> 12

Met Glu Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
1 5 10 15

Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
 20 25 30
 Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
 35 40 45
 Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Asp Glu
 50 55 60
 Asp Lys Arg Gly Tyr Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
 65 70 75 80
 Met Gln Ser Met Ile Lys Ala Met Asp Met Leu Lys Ile Ser Tyr Gly
 85 90 95
 Gln Gly Glu His Ser Glu Leu Ala Asp Leu Val Met Ser Ile Asp Tyr
 100 105 110
 Glu Thr Val Thr Thr Phe Glu Asp Pro Tyr Leu Asn Ala Ile Lys Thr
 115 120 125
 Leu Trp Asp Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
 130 135 140
 Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Ser Asp Leu Ala Arg
 145 150 155 160
 Ile Glu Gln Ala Asp Tyr Leu Pro Thr Glu Gln Asp Ile Leu Arg Ala
 165 170 175
 Arg Val Pro Thr Thr Gly Ile Leu Glu Tyr Pro Phe Asp Leu Asp Gly
 180 185 190
 Ile Val Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
 195 200 205
 Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
 210 215 220
 Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
 225 230 235 240
 Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
 245 250 255
 Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
 260 265 270

Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
275 280 285

Tyr Asp Gly Pro Lys Gln Asp His Ala Ala Ala Lys Gln Phe Val Leu
290 295 300

Lys Lys Tyr Leu Ala Cys Asn Pro Asp Pro Glu Arg Gln Cys Tyr Ser
305 310 315 320

His Phe Thr Thr Ala Thr Asp Thr Glu Asn Ile Lys Leu Val Phe Cys
325 330 335

Ala Val Lys Asp Thr Ile Met Gln Asn Ala Leu Lys Glu Phe Asn Leu
340 345 350

Gly

<210> 13

<211> 353

<212> PRT

<213> *Drosophila melanogaster*

<400> 13

Met Glu Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
1 5 10 15

Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
20 25 30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
35 40 45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Asp Glu
50 55 60

Asp Lys Arg Gly Tyr Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
65 70 75 80

Met Gln Ser Met Ile Lys Ala Met Asp Met Leu Lys Ile Ser Tyr Gly
85 90 95

Gln Gly Glu His Ser Glu Leu Ala Asp Leu Val Met Ser Ile Asp Tyr
100 105 110

Glu Thr Val Thr Thr Phe Glu Asp Pro Tyr Leu Asn Ala Ile Lys Thr
115 120 125

Leu Trp Asp Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
 130 135 140
 Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Lys Asp Leu Asp Arg
 145 150 155 160
 Val Ala Gln Pro Ala Tyr Leu Pro Thr Glu Gln Asp Ile Leu Arg Val
 165 170 175
 Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Glu
 180 185 190
 Ile Arg Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
 195 200 205
 Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
 210 215 220
 Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
 225 230 235 240
 Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
 245 250 255
 Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
 260 265 270
 Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
 275 280 285
 Tyr Asp Gly Pro Gln Arg Asp Ala Ile Thr Ala Arg Glu Phe Ile Leu
 290 295 300
 Arg Met Phe Val Asp Leu Asn Pro Asp Ser Glu Lys Ile Ile Tyr Ser
 305 310 315 320
 His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
 325 330 335
 Ala Val Lys Asp Thr Ile Leu Gln Ser Asn Leu Lys Glu Tyr Asn Leu
 340 345 350
 Val

<210> 14

<211> 353

<212> PRT

<213> Homarus americanus

<400> 14

Met Ala Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
1 5 10 15

Gln Glu Ile Glu Arg Gln Leu Arg Lys Asp Lys Arg Asp Ala Arg Arg
20 25 30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
35 40 45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ala Gly Tyr Ser Asp Glu
50 55 60

Asp Lys Arg Gly Phe Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
65 70 75 80

Met Gln Ser Met Ile Arg Ala Met Asp Leu Leu Gln Ile Ser Tyr Gly
85 90 95

Asp Ser Ala Asn Ile Glu His Ala Asp Leu Val Arg Ser Val Asp Tyr
100 105 110

Glu Ser Val Thr Thr Phe Glu Glu Pro Tyr Val Thr Ala Met Asn Ser
115 120 125

Leu Trp Gln Asp Thr Gly Ile Gln His Cys Tyr Asp Arg Arg Arg Glu
130 135 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Thr Asp Leu Asp Arg
145 150 155 160

Ile Ala Ala Lys Asp Tyr Val Ser Thr Leu Gln Asp Ile Leu Arg Val
165 170 175

Arg Ala Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Glu
180 185 190

Ile Arg Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
195 200 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
210 215 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn

Met	Gln	Ser	Met	Asn	Lys	Ala	Met	Glu	Met	Leu	Lys	Ile	Ser	Tyr	Lys	85	90	95
Asp	Arg	Asn	Asn	Ile	Glu	Asn	Ala	Glu	Leu	Val	Leu	Ser	Val	Asp	Tyr	100	105	110
Glu	Thr	Val	Thr	Thr	Phe	Asp	Ser	Pro	Tyr	Val	Glu	Ala	Ile	Lys	Ser	115	120	125
Leu	Trp	Val	Asp	Pro	Gly	Ile	Gln	Glu	Cys	Tyr	Asp	Arg	Arg	Arg	Glu	130	135	140
Tyr	Gln	Leu	Thr	Asp	Ser	Ala	Lys	Tyr	Tyr	Leu	Asn	Asp	Ile	Asp	Arg	145	150	155
Ile	Ala	Val	Pro	Asn	Tyr	Leu	Pro	Thr	Gln	Gln	Asp	Ile	Leu	Arg	Val	165	170	175
Arg	Val	Pro	Thr	Thr	Gly	Ile	Ile	Glu	Tyr	Pro	Phe	Ile	Leu	Asp	Ser	180	185	190
Ile	Ile	Phe	Arg	Met	Val	Asp	Val	Gly	Gly	Gln	Arg	Ser	Glu	Arg	Arg	195	200	205
Lys	Trp	Ile	His	Cys	Phe	Glu	Asn	Val	Thr	Ser	Ile	Ile	Phe	Leu	Val	210	215	220
Ala	Leu	Ser	Glu	Tyr	Asp	Gln	Ile	Leu	Phe	Glu	Ser	Asp	Asn	Glu	Asn	225	230	235
Arg	Met	Glu	Glu	Ser	Lys	Ala	Leu	Phe	Lys	Thr	Ile	Ile	Thr	Tyr	Pro	245	250	255
Trp	Phe	Leu	Asn	Ser	Ser	Val	Ile	Leu	Phe	Leu	Asn	Lys	Lys	Asp	Leu	260	265	270
Leu	Glu	Glu	Lys	Ile	Met	Phe	Ser	His	Leu	Val	Asp	Tyr	Phe	Pro	Glu	275	280	285
Tyr	Asp	Gly	Pro	Lys	Lys	Asp	Ala	Val	Gln	Gly	Arg	Glu	Phe	Ile	Leu	290	295	300
Lys	Met	Phe	Val	Asp	Leu	Asn	Pro	Asp	Ser	Glu	Lys	Ile	Ile	Tyr	Ser	305	310	315
His	Phe	Thr	Cys	Ala	Thr	Asp	Thr	Glu	Asn	Ile	Arg	Phe	Val	Phe	Ala	325	330	335

Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn Leu
 340 345 350

Val

<210> 16
 <211> 354
 <212> PRT
 <213> Loligo forbesi

<400> 16
 Met Ala Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
 1 5 10 15

Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
 20 25 30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
 35 40 45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Glu Glu
 50 55 60

Asp Arg Lys Gly Phe Glu Lys Ile Val Tyr Gln Asn Ile Phe Ser Ala
 65 70 75 80

Ile Gln Thr Leu Ile Ala Ala Met Glu Thr Leu Ser Leu Glu Tyr Lys
 85 90 95

Asp Pro Ser Asn Asn Glu His Ala Glu Phe Leu Asn Ser Ile Asp Ala
 100 105 110

Asp Ser Ala Asp Ile Phe Glu Asp Gly His Val Thr Ala Ile Lys Gly
 115 120 125

Cys Trp Thr Asp Pro Gly Met Gln Glu Cys Tyr Asp Arg Arg Arg Glu
 130 135 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Asp Asp Val Glu Arg
 145 150 155 160

Ile His Glu Pro Gly Tyr Ile Pro Thr Leu Gln Asp Ile Leu Arg Val
 165 170 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Tyr Ser
 180 185 190

Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
 195 200 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe Leu Val
 210 215 220

Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Ser Asp Asn Glu Glu
 225 230 235 240

Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr
 245 250 255

Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp
 260 265 270

Leu Leu Glu Glu Lys Ile Met Thr Ser His Leu Ala Asp Tyr Phe Pro
 275 280 285

Asp Tyr Asp Gly Pro Lys Cys Asp Tyr Glu Ala Ala Arg Glu Phe Met
 290 295 300

Met Asp Ser Tyr Met Asp Leu Asn Glu Asp Lys Glu Lys Met Leu Tyr
 305 310 315 320

Tyr His Tyr Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe
 325 330 335

Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn
 340 345 350

Leu Val

<210> 17

<211> 355

<212> PRT

<213> Caenorhabditis elegans

<400> 17

Met Ala Cys Cys Leu Ser Glu Glu Ala Arg Glu Gln Lys Arg Ile Asn
 1 5 10 15

Gln Glu Ile Glu Lys Gln Leu Gln Arg Asp Lys Arg Asn Ala Arg Arg
 20 25 30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr

290 295 300
 Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ala Asp Lys Ile Ile
 305 310 315 320
 Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val
 325 330 335
 Phe Ala Ala Val Lys Asp Thr Ile Leu Gln His Asn Leu Lys Glu Tyr
 340 345 350
 Asn Leu Val
 355

<210> 18
 <211> 355
 <212> PRT
 <213> Geodia cydonium

<400> 18
 Met Ser Cys Leu Leu Ser Glu Glu Glu Arg Leu Gln Lys Arg Ile Asn
 1 5 10 15
 Thr Arg Ile Asn Arg Glu Leu Gln Arg Asp His Lys Asp Ala Lys Lys
 20 25 30
 Glu Ile Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
 35 40 45
 Phe Ile Lys Gln Met Arg Ile Ile His Gly Lys Gly Tyr Ser Lys Gln
 50 55 60
 Asp Cys Leu Glu Tyr Lys Asn Leu Val Phe Arg Asn Ile Leu Met Ser
 65 70 75 80
 Met His Ser Met Leu Gln Ala Thr Ala Glu Leu Lys Ile Ala Tyr Ile
 85 90 95
 Asp Pro Asp Ala Gln Arg His Val Gln Leu Leu Met Ala Leu Arg Pro
 100 105 110
 Glu Thr Ala Gln Ser Leu Gly Gly Glu Thr Cys Glu Ala Ile Arg Lys
 115 120 125
 Leu Trp Gln Asp Ala Gly Val Gln Glu Cys Tyr Gln Arg Arg Asn Glu
 130 135 140

Tyr	Gln	Leu	Ser	Asp	Ser	Thr	Lys	Tyr	Tyr	Leu	Asp	Asp	Leu	Pro	Arg	145	150	155	160
Ile	Ser	Ser	Asn	Asp	Tyr	Val	Pro	Thr	Thr	Gln	Asp	Val	Leu	Arg	Val	165	170	175	
Arg	Val	Pro	Thr	Thr	Gly	Ile	Asn	Glu	Tyr	Pro	Phe	Thr	Ile	Asn	Lys	180	185	190	
Ile	Ile	Phe	Lys	Met	Val	Asp	Val	Gly	Gly	Gln	Arg	Ser	Glu	Arg	Arg	195	200	205	
Lys	Trp	Ile	His	Cys	Phe	Asp	His	Val	Thr	Ser	Val	Met	Phe	Leu	Val	210	215	220	
Ala	Ile	Ser	Glu	Tyr	Asp	Gln	Ile	Leu	Val	Glu	Ala	Asp	Ser	Arg	Val	225	230	235	240
Asn	Arg	Met	Val	Glu	Ser	Leu	His	Leu	Phe	Asn	Thr	Ile	Ile	Ser	Tyr	245	250	255	
Pro	Trp	Phe	Asn	Lys	Ser	Ser	Ile	Ile	Leu	Phe	Leu	Asn	Lys	Lys	Asp	260	265	270	
Leu	Leu	Glu	Glu	Lys	Val	Met	His	Ser	His	Leu	Ile	Asp	Tyr	Phe	Glu	275	280	285	
Glu	Tyr	Asp	Gly	Pro	Lys	Cys	Asp	His	Val	Ser	Ala	Arg	Glu	Ser	Ile	290	295	300	
Ala	Lys	Met	Phe	Ile	Ser	Ile	Asn	Asp	Met	Arg	Ser	Ala	Asp	Ile	Tyr	305	310	315	320
Pro	His	Phe	Thr	Cys	Ala	Thr	Asp	Thr	Glu	Asn	Ile	Lys	Phe	Val	Phe	325	330	335	
Asp	Val	Val	Lys	Asn	His	Ile	Leu	Gln	Gln	His	Ile	Thr	Glu	Val	Val	340	345	350	
Pro	Gly	Leu														355			

<210> 19
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 19

gaatatgatg gaccccagag agatg

25

<210> 20

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 20

gacacctgag ttagcacagt ccgatgtact tcaggttcaa ctggaggatg gt

52

<210> 21

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 21

gacacctgag ttagtacagt ccgcattcct tcaggttcaa ctggaggatg gt

52

<210> 22

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 22

gacacctgag ttagtaaagc ccacattcct tcaggttcaa ctggaggatg gt

52

<210> 23

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 23

gatactcgag ttagagcagc tcgtattgct tcaggttcaa ctggaggatg gt 52

<210> 24

<211> 58

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 24

ggaaaaaagc ggccgcttaa aacagtccgc agtcottcag gttcaactgg aggatggg 58

<210> 25

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 25

gggggtaccgc cgccatggcc tgctgtttat cc 32

<210> 26

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 26

gctctagatt acaccaagtt gtactccttc agatt 35

<210> 27

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 27

ctctccgata tccgacggct g

21

<210> 28

<211> 64

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 28

ttctacagca taatctgaag tatatcggtt tgtgttaata tagagggccc gtttaaacc 60
gctg 64

<210> 29

<211> 64

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 29

cagcgggttt aaacggggccc tctagattaa cacaaaccga tatacttcag attatgctgt 60
agaa 64

<210> 30

<211> 46

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 30

cagcataatc tgaaggagtg tggattgtac taatctagag ggcccc 46

<210> 31

<211> 46

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 31

cgggccctct agattagtag aatccacact ccttcagatt atgctg

46

<210> 32

<211> 69

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 32

ggaaaaaagc ggccgcttag agcagctcgt attgcctcag gtgcattctgg aggatgggtg 60

ccttgacgg

69

<210> 33

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 33

gctctagatt agagcagctc gtattgcctc aggtgcatct gtagaattgt gtctttgacg 60

gcg

63

<210> 34

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 34

gctctagatt aacatagccc tatgtatttt agattattct gtagaattgt gtctttgacg 60

gcg

63

<210> 35
<211> 98
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 35
gctctagatt agagcagctc gtattgcctc aggtgcatac gttgaataat gtcacgacag 60
tcattaaaaa cagccgaat gttttccgta tcagtcgc 98

<210> 36
<211> 6
<212> PRT
<213> vertebrate

<400> 36
Met Thr Leu Glu Ser Ile
1 5

<210> 37
<211> 21
<212> PRT
<213> invertebrate

<400> 37
Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln His Asn Leu Lys
1 5 10 15

Glu Tyr Asn Leu Val
20

<210> 38
<211> 21
<212> PRT
<213> vertebrate

<400> 38
Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Gln Asn Asn Leu Lys
1 5 10 15

Tyr Ile Gly Leu Cys
20

<210> 39
 <211> 21
 <212> PRT
 <213> vertebrate

<400> 39
 Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln Arg Met His Leu Arg
 1 5 10 15

 Gln Tyr Glu Leu Leu
 20

<210> 40
 <211> 21
 <212> PRT
 <213> vertebrate

<400> 40
 Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys
 1 5 10 15

 Glu Cys Gly Leu Tyr
 20

<210> 41
 <211> 353
 <212> PRT
 <213> Drosophila melanogaster

<400> 41
 Met Glu Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
 1 5 10 15

 Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
 20 25 30

 Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
 35 40 45

 Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Asp Glu
 50 55 60

 Asp Lys Arg Gly Tyr Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
 65 70 75 80

Met Gln Ser Met Ile Lys Ala Met Asp Met Leu Lys Ile Ser Tyr Gly
 85 90 95

Gln Gly Glu His Ser Glu Leu Ala Asp Leu Val Met Ser Ile Asp Tyr
 100 105 110

Glu Thr Val Thr Thr Phe Glu Asp Pro Tyr Leu Asn Ala Ile Lys Thr
 115 120 125

Leu Trp Asp Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
 130 135 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Lys Asp Leu Asp Arg
 145 150 155 160

Val Ala Gln Pro Ala Tyr Leu Pro Thr Glu Gln Asp Ile Leu Arg Val
 165 170 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Glu
 180 185 190

Ile Arg Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
 195 200 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
 210 215 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
 225 230 235 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
 245 250 255

Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
 260 265 270

Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
 275 280 285

Tyr Asp Gly Pro Gln Arg Asp Ala Ile Thr Ala Arg Glu Phe Ile Leu
 290 295 300

Arg Met Phe Val Asp Leu Asn Pro Asp Ser Glu Lys Ile Ile Tyr Ser
 305 310 315 320

His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
 325 330 335

Ala Val Lys Asp Thr Ile Leu Gln Ser Asn Leu Lys Tyr Ile Gly Leu
340 345 350

Cys

<210> 42

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 42

cggggtaccc cggttagcat ggagtgctgt ttatcg 36

<210> 43

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 43

ccggaattcc ggtagacca aattatattc cttaagggtc 40

<210> 44

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 44

gagcatcgat tacgagaccg ttacc 25

<210> 45

<211> 53

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 45

cggaattctt agcacagtcc gatgtactta aggttcgatt gcagaattgt gtc

53